October 1990
in two parts, part 1
volume 163, *number* 4

CUK-H01371-34-P024292

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby–Year Book, Inc.

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editors
ALBERT B. GERBIE
STEVEN G. GABBE
THOMAS J. GARITE
MOON H. KIM
ALBERTO MANETTA

34

Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

MOSBY–YEAR BOOK, INC.
St. Louis, Missouri 63146-3318

ISSN 0002-9378

Going.

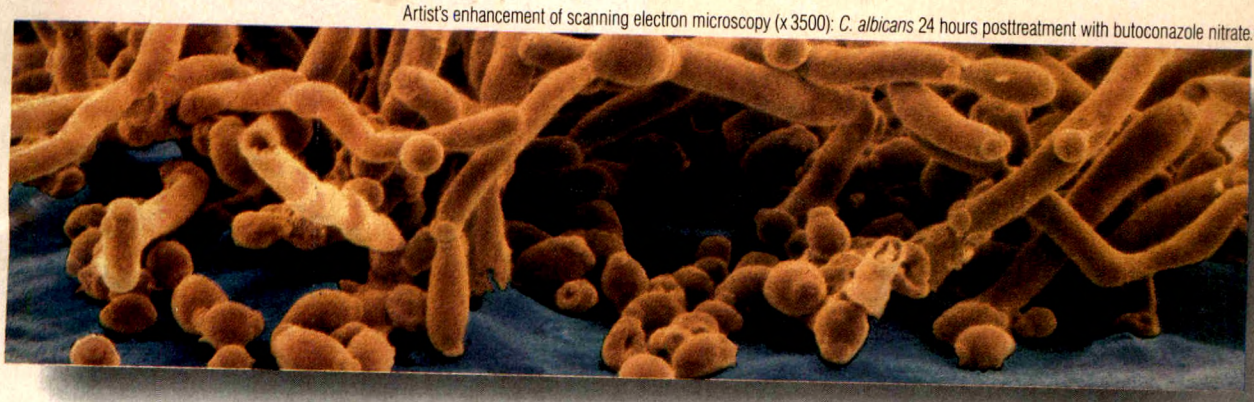
Going.

Gone.

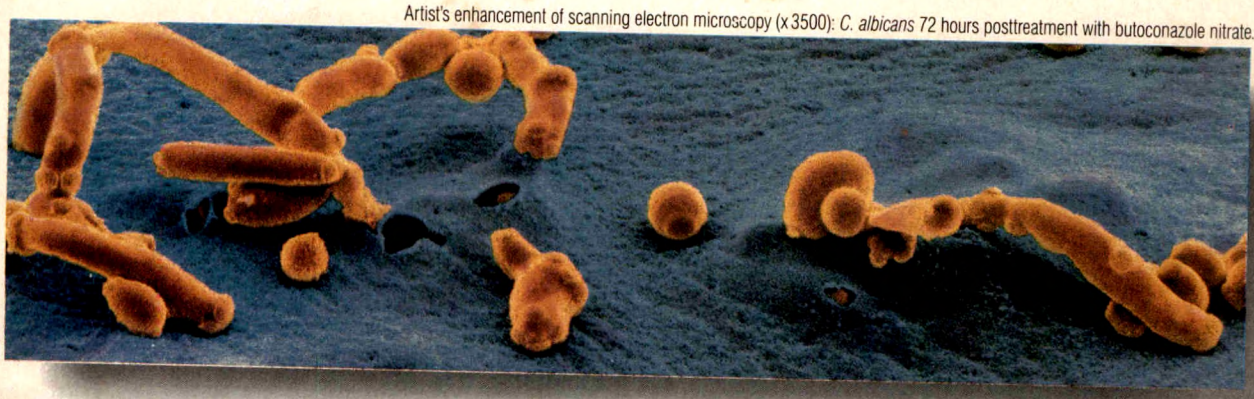
In vulvovaginal candidiasis, **the proven efficacy of 3-day therapy* with FEMSTAT Prefill means a microbiological cure in 95% of patients.[1†]**

1. Droegemueller W, Adamson DG, Brown D, et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64:530-534. *In pregnant patients (second and third trimesters only), therapy should be extended to six days. †Microbiological cure in 95% of patients (n = 126) eight days following FEMSTAT 3-day therapy. ‡ Micrographs on file, Syntex Laboratories, Inc. Please see next page for brief summary of prescribing information. © 1990 Syntex Laboratories, Inc. 813-H2-166-90

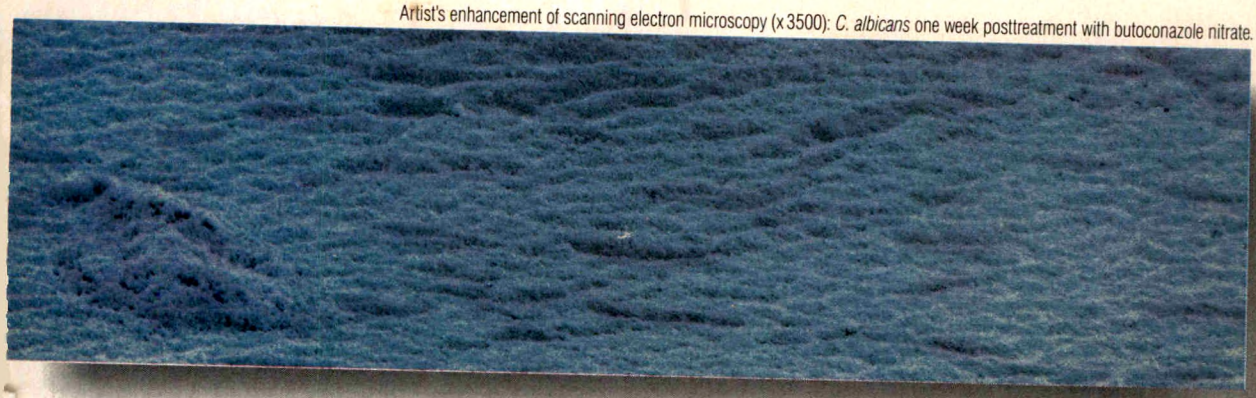
Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 24 hours posttreatment with butoconazole nitrate.‡



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 72 hours posttreatment with butoconazole nitrate.



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* one week posttreatment with butoconazole nitrate.



P 24292

34

FEMSTAT® PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%

So effective, it works with 3-day therapy.



SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Committed to the OB/GYN and women's healthcare

CABGUTTA
CENTRAL LIBRARY

FEMSTAT PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%

BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

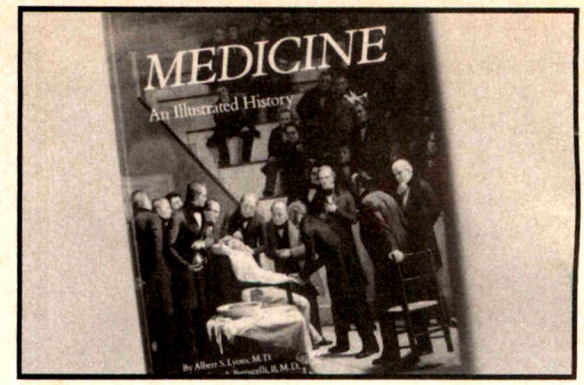
DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304



MEDICINE: An Illustrated History

By Albert S. Lyons, M.D., F.A.C.S. and R. Joseph Petrucelli II, M.D.; with 14 contributors

This splendid survey will provide unending hours of pleasure. You'll enjoy relaxing with it after long hours in the office.

- brilliant color pictures review every dimension of medical history
- historical breakthroughs in medicine recorded with spellbinding prose
- timeless classic makes a splendid gift

1974. 618 pages, 1,020 illustrations including 250 in full color (Book Code: 03374) $42.95 (U.S.); $56.00 (Can.)

Save time! Call toll-free: 800-426-4545, Monday-Friday, 7AM-7PM, Central Time FAX orders, 314-432-1380 Attn: Direct Marketing Please mention this number when calling: FLR-619-999-03 In Canada, call collect: 416-298-2465 For Customer Service: 800-633-6699 Monday-Friday, 7AM-6PM, Central Time

Mosby-Year Book • 11830 Westline Industrial Drive • St. Louis, MO 63146

Mosby-Year Book, Ltd. • 5240 Finch Avenue East • Scarborough, Ontario • Canada M1S 5A2

YES! Please send ____ copies of (03374) Lyons, MEDICINE: An Illustrated History, priced at $42.95 (U.S.); $56.00 (Can.), on no-risk, 30-day approval.

SAVE MONEY! Send a check with your order or charge it to your credit card. Mosby-Year Book pays the postage on all prepaid orders. Please add $3.50 per order to cover handling charges. Other orders will be billed for postage and handling.

☐ Payment enclosed

☐ Bill me, plus postage and handling

Charge my: ☐ MasterCard ☐ VISA ☐ AmEx

Card # _____ Exp. date _____

Signature _____

Business phone (_____) _____

Name _____

Address _____

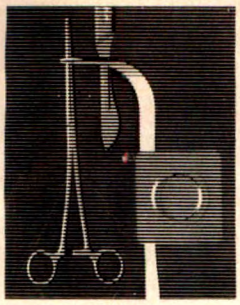
City _____

State _____ Zip _____

If using a purchase order, please attach this coupon and send to Pat Newman at the Mosby-Year Book address. Outside the U.S. and Canada, please pay by check or money order in dollar equivalent. 30-day approval good in U.S. and Canada. Prices subject to change without notice. Add applicable sales tax.

FLR-619-999-03

Mosby Year Book



Count on

CEFOTAN® IM/IV
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli*.
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli*.
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

Females: 0.9 × value for males

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

Manufactured for

STUART PHARMACEUTICALS
A business unit of ICI Americas Inc.
Wilmington, Delaware 19897 USA

Rev. I 11/88 63985-01

Cefotan arithmetic.

CEFOTAN IV 2g, q12h + doxycycline IV or PO 100 mg, q12h

•CDC• CENTERS FOR DISEASE CONTROL RECOMMENDED FOR PID[7]

more cost-effective than cefoxitin[1,2]

less frequent dosing than cefoxitin[3,4]

several times the half-life of cefoxitin[5,6]

single-dose prophylaxis/ twice-daily treatment



Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

© 1989 ICI America

Mosby Year Book

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1990 by Mosby–Year Book, Inc.

Contents

October
Part 1
1990

EDITORIAL

The first seventy years 1109

CLINICAL SECTION

Clinical Opinion

Drug prophylaxis for human immunodeficiency virus–infected pregnant women: Ethical considerations 1111

Howard L. Minkoff, MD, and Jonathan D. Moreno, PhD

Brooklyn, New York

The appropriateness of using new human immunodeficiency virus therapeutic agents, with unknown fetal effects, in the antepartum period is discussed.

Efficacy and safety considerations in women with uterine leiomyomas treated with gonadotropin-releasing hormone agonists: The estrogen threshold hypothesis 1114

Andrew J. Friedman, MD, Susan M. Lobel, MD, Mitchell S. Rein, MD, and Robert L. Barbieri, MD

Boston, Massachusetts

Treatment of women with uterine leiomyomas with gonadotropin-releasing hormone agonists may be useful preoperatively; the addition of low doses of steroid hormones to gonadotropin-releasing hormone agonist therapy may be useful for long-term medical treatment.

Clinical Articles

Contraception and ectopic pregnancy risk 1120

Adele L. Franks, MD, Valerie Beral, MB, BS, Willard Cates, Jr., MD, MPH, and Carol J. R. Hogue, PhD

Atlanta, Georgia

To clarify the relationship between contraception and ectopic pregnancy, we estimated incidence rates of ectopic pregnancy for a variety of contraceptive methods.

(Contents continued on page 7A)

Vol. 163, No. 4, Part 1, October 1990. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 7351. Annual subscription rates for 1991: domestic, $86.00 for individuals and $150.00 for institutions. Printed in the U.S.A.
Copyright © 1990 by Mosby–Year Book, Inc.

SonoVu US™—A visible improvement in ultrasound-guided aspiration.

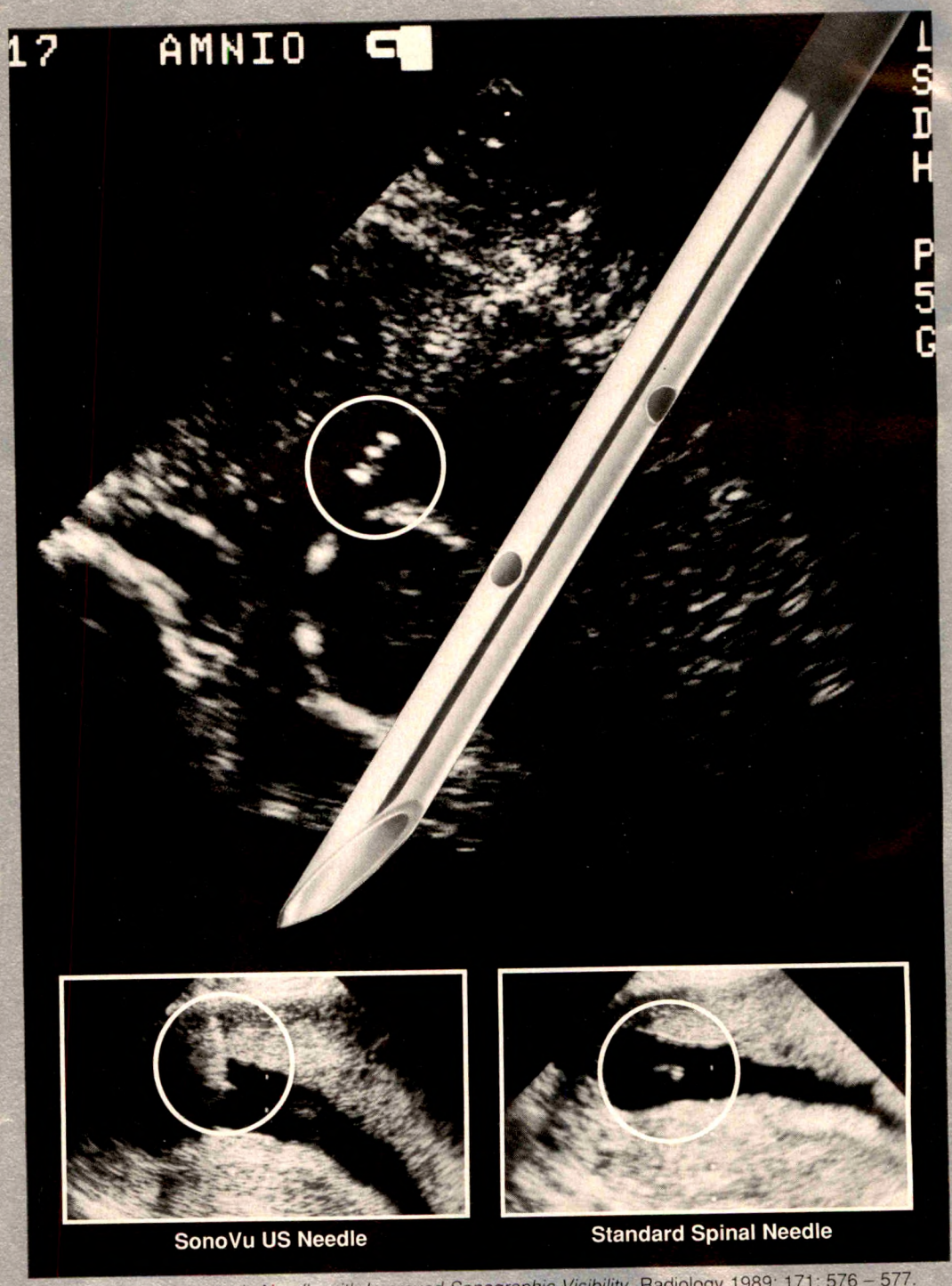
The clearly visible bright echoes on the real-time sonogram below mark a breakthrough in accuracy and safety for fine-needle aspiration under ultrasound guidance.

SonoVu US, from E-Z-EM, is the first in a family of procedural needles designed specifically for enhanced ultrasound visualization.

Precisely formed 0.44 mm distal side holes on the cannula closely match the wavelength of ultrasound generated by a 3.5 MHz transducer. Uniform diffraction of the wave at each hole creates a highly visible echo on the US display.

The side holes also provide a significant increase in needle flow rate, and ensure free flow should the tip become occluded—often the case with spinal or chiba needles.

Developed for use in amniocentesis, the SonoVu design can offer similar benefits in such procedures as US-monitored biopsy and cyst aspiration. For more information on 20- and 22-gauge SonoVu US needles from E-Z-EM, call 1-800-544-4624.



For both the patient and the practitioner, E-Z-EM products set the standards for convenience, safety, and quality in:

- GI Radiology
- Interventional Radiology
- CT Scanning
- Ultrasound
- Magnetic Resonance Imaging
- Angiography
- CEA Immunostaining
- Radiation Oncology
- Patient Prepping
- Protective Products
- Computerized Information Management Systems

E-Z-EM®

E-Z-EM, Inc.
Westbury, NY 11590

© 1990, E-Z-EM, Inc.

Reference: *Amniocentesis Needle with Improved Sonographic Visibility*. Radiology 1989; 171: 576 – 577.

Contents continued from page 5A

Calcium supplementation during pregnancy may reduce preterm delivery in high-risk populations 1124
Jose Villar, MD, and John T. Repke, MD
Bethesda and Baltimore, Maryland

Calcium supplementation in a high-risk population lowers blood pressure and may reduce the incidence of preeclampsia and prematurity.

The association of intrapartum asphyxia in the mature fetus with newborn behavior 1131
James A. Low, MD, Darwin W. Muir, PhD, Elizabeth A. Pater, RN, and E. Jane Karchmar, BA
Kingston, Ontario, Canada

Many newborns who have undergone an intrapartum asphyxial insult will not have evidence of central nervous system injury.

Sexually transmitted diseases and human immunodeficiency virus infection among women with pelvic inflammatory disease 1135
Bente Hoegsberg, MD, Ovadia Abulafia, MD, Alexander Sedlis, MD, Joseph Feldman, DrPH, Don DesJalais, PhD, Sheldon Landesman, MD, and Howard Minkoff, MD
Brooklyn and New York, New York

Women hospitalized with pelvic inflammatory disease and human immunodeficiency virus infection (15 of 110, 13.6%) had a hospital course similar to that of human immunodeficiency virus–negative women with a trend toward more operative interventions.

The effect of large uterine fibroids on urinary bladder function and symptoms 1139
Rami Langer, MD, Abraham Golan, MD, Menachem Neuman, MD, David Schneider, MD, Ian Bukovsky, MD, and Eliahu Caspi, MD
Zerifin, Israel

Bladder symptoms were lessened after reduction of uterine fibroids by gonadotropin-releasing hormone analogs.

(Contents continued on page 9A)

1991 Annual subscription rates	U.S.A.	*International (surface mail)* *All regions*	*International (airmail)**				
			Canada	*Mexico*	*Region 1*	*Region 2*	*Region 3*
Institutions†	$150.00	$182.00	$215.00	$223.00	$238.25	$290.50	$342.50
Individuals‡	$ 86.00	$118.00	$151.00	$159.00	$174.25	$226.50	$278.50
Students, residents‡	$ 40.00	$ 72.00	$105.00	$113.00	$128.25	$180.50	$232.50

Subscription rates include supplements. Single copies are $8.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

**Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

THE PERMANENTE MEDICAL GROUP

NORTHERN CALIFORNIA



The Permanente Medical Group, the largest multispecialty group practice in the U.S., is undergoing explosive growth in its Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley. Our rapid increase in membership has created practice opportunities throughout the region for additional BC/BE Obstetricians and Gynecologists.

Our physician-managed group is part of the comprehensive Kaiser Permanente Medical Care Program. As a TPMG physician, you have access to the latest medical technology and resources, the support of colleagues in all subspecialties — and the opportunity to provide excellent health care without the burdens of managing a practice.

Join us in one of these facilities:

QUALITY

STABILITY

LEADERSHIP

GROWTH

SUPPORT

REWARDS

Richmond — Small, congenial clinic setting staffed by five physicians and five nurse practitioners with ready consultation for other services, including perinatology. Practice general OB/GYN, high-risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser welcome. Resident teaching opportunities exist. Staff call at Kaiser Hospital in Oakland, shared with resident staff.

Oakland — Twenty physicians, 12 residents offer services in oncology, infertility, maternal-fetal medicine and a large ICN.

Redwood City — Specializes in family-centered maternity care; offers modern LDR rooms and full range of gynecological surgery. With ten physicians, 24-hour call is required three times each month. Beautiful Peninsula location, near San Francisco and the Pacific Ocean.

Stockton — Small four physician department in a new clinic building with new equipment, including laser. Stockton's lifestyle is affordable and attractive, offering recreational activities such as boating on the inland waterways. Centrally located, with mountains to the east and San Francisco Bay Area to the west.

Roseville — Eight physicians and two nurse practitioners in clinic setting. Patients are hospitalized for delivery and surgery in Sacramento where physicians take call for L&D and emergency about 5 times each month.

San Francisco — Full range of services including large high-risk, tertiary care OB service. Eighteen physicians, including GYN Oncologist. Residency program. Clinical research and academic teaching appointments available.

Sacramento — Growing metropolitan area between San Francisco Bay Area and Sierra Nevada mountains. Up-to-date facility with active staff of 14 and excellent residency program affiliated with the University of California at Davis. Flexible call averaging five shifts per month covers L&D, emergency room and inpatient services.

Pleasanton — Staffed by four physicians and three nurse practitioners, clinic provides general OB/GYN services, non-stress tests, ultrasound and cystoscopy. Consultant support, including a Perinatologist, readily available. Share call with staff of Walnut Creek Medical Center. Affiliated with the University of California at San Francisco as clinical instructors.

Fresno — Ground-floor opportunities in growing area for clinically-oriented individuals. Provide general OB/GYN; special interests encouraged. Planning new $26 million outpatient facility to accompany current medical office building. On call every fourth night and weekend. Local residency program at Valley Medical Center affiliated with the University of California at San Francisco. Teaching positions possible.

South San Francisco — Full service except L&D. Nine physicians, six nurse practitioners. On San Francisco Peninsula, 15 minutes from downtown.

KAISER PERMANENTE
Good People. Good Medicine.

TPMG offers many benefits: scheduled time off with cross-coverage provided by your colleagues, continuing education, malpractice insurance, a substantial retirement program and special arrangements for physicians transferring from established practice. For complete information, call or send CV to: The Permanente Medical Group, Inc., Richmond Prescott, M.D., Physician Recruitment Services, Dept. AOG-8348, 1814 Franklin, 4th Floor, Oakland, CA 94612. (800) 777-4912. EOE

Contents continued from page 7A

The natural history of thrombocytopenia associated with preeclampsia 1142
Vern L. Katz, MD, John M. Thorp, Jr., MD, Lynda Rozas, BA, and Watson A. Bowes, Jr., MD
Chapel Hill, North Carolina

Thrombocytopenia associated with preeclampsia should resolve by postpartum day 4.

Continuing controversy in alloimmune thrombocytopenia: Fetal hyperimmunoglobulinemia fails to prevent thrombocytopenia 1144
Umberto Nicolini, MD, Yuen Tannirandorn, MD, Pedro Gonzalez, MD, Nicholas M. Fisk, MD, Jeremy Beacham, MSc, Elizabeth A. Letsky, MD, and Charles H. Rodeck, MD
London, England

Weekly in utero platelet transfusions are the only treatment that guarantees a safe fetal platelet count in cases of severe alloimmune thrombocytopenia.

Low fetal risks in pregnancies associated with idiopathic thrombocytopenic purpura 1147
Robert F. Burrows, MD, and John G. Kelton, MD
Hamilton, Ontario, Canada

Because only 3 of 61 infants of mothers with idiopathic thrombocytopenic purpura had a cord platelet count of $<50 \times 10^9$/L, maternal interventions that lead to maternal morbidity cannot be justified.

Laparoscopic ventrosuspensions 1151
Ann F. E. Yoong, MB, ChB
Merseyside, England

Laparoscopic ventrosuspension does not appear to be helpful in the management of deep dyspareunia or pelvic pain in women with retroverted uteri.

Pregnancy-induced hypertension and acute fatty liver of pregnancy: Atypical presentations 1154
Mark A. Brown, MD, George Passaris, MB, BS, and Mark A. Carlton, MB, BS
Sydney, Australia

Acute fatty liver of pregnancy and pregnancy-induced hypertension form a clinical continuum, and acute fatty liver of pregnancy may be seen with an isolated increase in hepatic alkaline phosphatase.

Vibroacoustic stimulation and fetal behavioral state in normal term human pregnancy 1156
Lawrence D. Devoe, MD, Cheri Murray, RN, David Faircloth, BS, and Edgard Ramos, MD
Augusta, Georgia

Vibroacoustic stimulation of normal term human fetuses resulted in alterations of behavioral state that appeared to be related to prestimulus behavioral state.

(Contents continued on page 10A)

Contents continued from page 9A

Successful conservative management of primary nonmetastatic cervical choriocarcinoma 1161
Avraham Ben-Chetrit, MD, Simcha Yagel, MD, Ilana Ariel, MD, David Zacut, MD, Shlomo Shimonovitz, MD, and Drorit Celnikier-Hochner, MD
Jerusalem, Israel

Primary cervical choriocarcinoma that occurred in a young patient who desired to preserve her fertility was successfully treated by local excision of the tumor followed by chemotherapy.

The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemotherapy after surgical cytoreduction 1164
Verda J. Hunter, MD, Lee Daly, PA-C, Michael Helms, PhD, John T. Soper, MD, Andrew Berchuck, MD, Daniel L. Clarke-Pearson, MD, and Robert C. Bast, Jr., MD
Durham, North Carolina

A CA 125 half-life of <20 days during primary chemotherapy was associated with prolonged survival in patients with advanced epithelial ovarian cancer.

Management and outcomes of pregnancies complicated by human B19 parvovirus infection: A prospective study 1168
John F. Rodis, MD, Dorothy L. Quinn, MS, G. William Gary, Jr., MPH, DrPH, Larry J. Anderson, MD, Sally Rosengren, MD, Matthew L. Cartter, MD, Winston A. Campbell, MD, and Anthony M. Vintzileos, MD
Farmington and Hartford, Connecticut, and Atlanta, Georgia

Human B19 parvovirus infection in pregnancy is associated with a fetal loss rate of 5%.

Colon cancer in pregnancy with elevated maternal serum α-fetoprotein level at presentation 1172
Whitney Gonsoulin, MD, Brian Mason, MD, and Robert J. Carpenter, Jr., MD
Houston, Texas

A case of colon cancer first seen with elevated maternal serum α-fetoprotein levels in described.

Effects of hypertension on pregnancy monitoring and results 1173
Jack W. Lenox, MD, Valentine Uguru, MD, and Luis A. Cibils, MD
Chicago, Illinois

A 2-year review of patients with hypertension reveals that a six-base line in late decelerations is a good predictor of fetal outcome.

Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis 1180
Cynthia Chazotte, MD, Barbara Girz, DO, Mordecai Koenigsberg, MD, and Wayne R. Cohen, MD
Bronx, New York

We report infarction of a large chorioangioma associated with regression of hydrops fetalis.

Contents

Ovarian pregnancy: A report of twenty cases in one institution 1182
Ariel Raziel, MD, Abraham Golan, MD, Mordechai Pansky, Md, Raphael Ron-El, MD, Ian Eukovsky, MD, and Eliahu Caspi, MD
Tel Aviv, Israel

Twenty cases of primary ovarian pregnancy diagnosed, treated, and followed up in one institution are presented; all the potentially fertile patients used an intrauterine contraceptive device.

Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21 1186
Norman Ginsberg, MD, Alan Cadkin, MD, Eugene Pergament, MD, PhD, and Yury Verlinsky, PhD
Chicago, Illinois

Biparietal diameter/femur length ratio and nuchal thickness are sensitive prenatal indicators of trisomy 18 and trisomy 21.

Second-trimester ultrasonography: Determinants of visualization of fetal anatomic structures 1191
Val Catanzarite, MD, PhD, and J. Gerald Quirk, MD, PhD
San Diego, California, and Little Rock, Arkansas

Maternal weight, gestational age, and maternal skin thickness were the major factors affecting visualization of fetal anatomic structures in a prospective series of 573 second-trimester ultrasonographic examinations.

Infertility and eating disorders 1196
Donna E. Stewart, MD, G. Erlick Robinson, MD, David S. Goldbloom, MD, and Charlene Wright, BSc
Toronto, Ontario, Canada

A previously undisclosed eating disorder (anorexia nervosa, bulimia nervosa, or eating disorder not otherwise specified) was discovered in 16.7% of consecutive infertility clinic patients.

Postpartum osteomyelitis caused by group B streptococcus 1200
K. Eerkowitz, MD, and R. McCaffrey, MD
New York, New York

Postpartum group B streptococcal osteomyelitis developed in a woman with vaginal colonization of group B streptococcus organisms and necessitated total hip replacement.

Antenatal spontaneous perforation of the extrahepatic biliary tree 1201
Syam Chilukuri, MD, Virgilio Bonet, MD, and Mason Cobb, MD
East Lansing, Michigan

Spontaneous perforation of the biliary duct can cause fetal ascites.

(Contents continued on page 12A)

Contents continued from page 11A

Antenatal diagnosis of molybdenum cofactor deficiency 1203
R. George F. Gray, PhD, Anne Green, MSc, Shishta Nadda Basu, MRCOG, Glyn Constantine, MD, Roy G. Condie, MD, Claude Dorche, PhD, Christine Vianey-Liaud, PhD, and Pierre Desjacques, PhD
Birmingham, United Kingdom, and Lyon, France

The assay of sulfite oxidase in chorionic villus tissue allows a rapid and early diagnosis of molybdenum cofactor deficiency.

Preoperative evaluation of serum CA 125, TAG 72, and CA 15-3 in patients with endometrial carcinoma 1204
John T. Soper, MD, Andrew Berchuck, MD, George J. Olt, MD, Andrew P. Soisson, MD, Daniel L. Clarke-Pearson, MD, and Robert C. Bast, Jr., MD
Durham, North Carolina

Tumor-associated antigen assays were not effective in detecting occult metastatic endometrial carcinoma.

Maternal serum α-fetoprotein levels in pregnancies complicated by diabetes: Implications for screening programs 1209
Alice O. Martin, PhD, Lisa M. Dempsey, MS, John Minogue, DMin, Kiang Liu, PhD, James Keller, MD, Ralph Tamura, MD, and Norbert Freinkel, MD
Chicago, Illinois

Maternal serum α-fetoprotein levels in diabetic patients are negatively associated with glycosylated hemoglobin values early in pregnancy and show a trend to be lower in the presence of early fetal growth delay.

High infectious morbidity in pregnant women with insulin-dependent diabetes: An understated complication 1217
Eric F. Stamler, MD, Maria L. Cruz, MD, Francis Mimouni, MD, Barak Rosenn, MD, Tariq Siddiqi, MD, Jane Khoury, MS, and Menachem Miodovnik, MD
Cincinnati, Ohio

Insulin-dependent diabetic pregnant patients are at significant risk for antepartum and postpartum infections; infectious morbidity may be associated with glycemic control.

The effect of race on the relationship between fetal death and altered fetal growth 1222
Richard Ferguson, MS, and Stephen A. Myers, DO
Chicago, Illinois

Outcome-derived risk assessment shows that 10th percentile birth weights do not consistently predict fetal death and that black fetuses are more sensitive to altered growth.

Effects of intravascular fetal blood transfusion on fetal intracardiac Doppler velocity waveforms 1231
Giuseppe Rizzo, MD, Kypros H. Nicolaides, MD, Domenico Arduini, MD, and Stuart Campbell, MD
Rome, Italy, and London, England

Intravascular fetal blood transfusion is associated with a temporary decrease in fetal cardiac output that is proportional to the degree of expansion in fetoplacental blood volume.

Contents

Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy 1239
Jim van Eyck, MD, PhD, Patricia A. Stewart, PhD, and Juriy W. Wladimiroff, MD, PhD
Rotterdam, The Netherlands

Averaged foramen ovale flow velocity increases during active sleep as compared with quiet sleep, suggesting increased right-to-left shunting at cardiac level.

Suppression of serum insulin level by diazoxide does not alter serum testosterone or sex hormone–binding globulin levels in healthy, nonobese women 1243
John E. Nestler, MD, Roshnara Singh, MD, Dennis W. Matt, PhD, John N. Clore, MD, and William G. Blackard, MD
Richmond, Virginia

Insulin suppression does not alter serum testosterone or sex hormone–binding globulin levels in nonobese, healthy women, suggesting that in these women insulin does not regulate testosterone metabolism.

Intrapartum uterine rupture and inflammatory bowel disease 1247
Ila M. Peterson, MD, and Bev Braak, MD
Kansas City, Missouri

This case report describes a previously undocumented association between steroid therapy for idiopathic inflammatory bowel disease and uterine rupture.

Maternal serum Down syndrome screening: Free β-protein is a more effective marker than human chorionic gonadotropin 1248
James N. Macri, PhD, Ramana V. Kasturi, BE, MS, David A. Krantz, BS, Edward J. Cook, PhD, Norman D. Moore, Jean A. Young, Kathleen Romero, BS, and John W. Larsen, Jr., MD
Carle Place, New York, and Washington, D.C.

Maternal serum screening for Down syndrome incorporating maternal age, α-fetoprotein, and the free β-protein of human chorionic gonadotropin can achieve 80% detection efficiency.

Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation from amniotic fluid 1253
Carl P. Weiner, MD, and Charles Grose, MD
Iowa City, Iowa

Congenital cytomegalovirus infection was diagnosed prenatally by virus isolation from the amniotic fluid of two pregnant women.

Pelvimetry by magnetic resonance imaging in breech presentation 1256
Aren J. van Loon, MD, Albert Mantingh, MD, PhD, Cornelis J. P. Thijn, MD, PhD, and Eduard L. Mooyaart, MD, PhD
Groningen, The Netherlands

Pelvimetry with magnetic resonance imaging provides a safe and reliable method for pelvic assessment in breech presentation.

(Contents continued on page 14A)

Contents continued from page 13A

Intraamniotic infection in the very early phase of the second trimester 1261
Israel Goldstein, MD, Etan Z. Zimmer, MD, David Merzbach, PhD, Bezaleal A. Peretz, MD, and Etan Paldi, MD
Haifa, Israel

Intraamniotic infection can exist early in pregnancy, even with intact membranes, and in most cases without any clinical symptoms.

Placental histology in fetuses between 18 and 23 weeks' gestation with abnormal karyotype 1264
R. S. Kuhlmann, MD, PhD, A. L. Werner, MD, J. Abramowicz, MD, S. L. Warsof, MD, J. Arrington, RDMS, and D. L. Levy, MD
Norfolk, Virginia

Undervascularization of the placenta is seen in pregnancies complicated by abnormal karyotype.

Carbon dioxide laser energy disperses human papillomavirus deoxyribonucleic acid onto treatment fields 1271
Alex Ferenczy, MD, Christine Bergeron, MD, PhD, and Ralph M. Richart, MD
Montreal, Quebec, Canada, and New York, New York

Human papillomavirus deoxyribonucleic acid is present in anogenital skin that has been treated by carbon dioxide laser vaporization.

Emergency treatment with recombinant tissue plasminogen activator of pulmonary embolism in a pregnant woman with antithrombin III deficiency 1274
F. Baudo, MD, T. M. Caimi, R. Redaelli, PhD, A. M. Nosari, MD, M. Mauri, MD, G. Leonardi, MD, and F. deCataldo, MD
Milan, Italy

A 29-year-old pregnant woman with congenital antithrombin III deficiency and pulmonary embolism was successfully treated with recombinant tissue plasminogen activator without treatment-related complications to the newborn.

Collagen metabolism in gynecologic patients: Changes in the concentration of the aminoterminal propeptide of type III procollagen in serum 1276
Ulla Puistola, MD, PhD, Leila Risteli, MD, PhD, Juha Risteli, MD, PhD, and Antti Kauppila, MD
Oulu, Finland

Collagen metabolism measured with serum aminoterminal propeptide of type III procollagen is increased during the luteal phase of the cycle and in severe salpingo-oophoritis.

Current Development

Why all placentas should be examined by a pathologist in 1990 1282
Carolyn M. Salafia, MD, and Anthony M. Vintzileos, MD
Farmington, Connecticut

The clinical correlates of placental lesions and their clinical, as well as mediocolegal, implications for obstetric-neonatal practice are discussed.

Contents

BASIC SCIENCE SECTION

Role of vasopressin in mediation of fetal cardiovascular responses to acute hypoxia 1294

Kathleen M. Piacquadio, MD, Robert A. Brace, PhD, and Cecilia Y. Cheung, PhD

La Jolla, California

Arginine vasopressin may mediate in part the fetal heart rate response to acute severe hypoxia but does not appear to regulate fetal blood volume, arterial pressure, or venous pressure responses to hypoxia.

Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions. III. Cytotoxic potential in vitro and characterization of antigen(s) 1301

William H. Kutteh, MD, PhD, Vicki V. Baker, MD, and George J. Doellgast, PhD

Birmingham, Alabama, and Winston-Salem, North Carolina

Autologous antibodies eluted from the cellular membrane fragments obtained from human ovarian neoplastic effusions are capable of complement-mediated cytotoxicity of human ovarian tumor cells.

Mechanism of action of interleukin-1β in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells 1307

Felice Petraglia, MD, Gian Carlo Garuti, MD, Biagina De Ramundo, MD, Stefano Angioni, MD, Andrea R. Genazzani, MD, and Louise M. Bilezikjian, PhD

Modena, Italy, and La Jolla, California

The addition of interleukin-1β increases corticotropin-releasing factor and adrenocorticotropin hormone release from cultured placental cells, acting in part via a prostaglandin pathway.

Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep 1313

Robert J. Morrow, MD, S. Lee Adamson, PhD, Shelley B. Bull, PhD, and J. W. Knox Ritchie, MD

Toronto, Ontario, Canada

Hypoxic acidosis, hyperviscosity, and maternal hypertension in fetal sheep do not cause umbilical arterial velocity waveform abnormalities similar to those of growth-restricted human fetuses.

A progesterone-induced blocking factor corrects high resorption rates in mice treated with antiprogesterone 1320

Julia Szekeres-Bartho, MD, PhD, G. Chaouat, MD, PhD, and R. Kinsky, DV, PhD

Pecs, Hungary, and Paris, France

Administration of preformed progesterone-induced blocking factor counteracted the abortive effect of progesterone receptor block.

(Contents continued on page 16A)

Contents continued from page 15A

Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy 1323
Kristjar Skajaa, MD, Danny Svane, MD, Karl-Erik Andersson, MD, PhD, and Axel Forman, MD, PhD
Arhus, Denmark, and Lund, Sweden

The thromboxane analog U46619 showed powerful vasoconstrictor effects in human maternal and fetal uteroplacental arteries; the responses could be inhibited by magnesium and the calcium antagonist isradipine.

The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes 1333
George Lewandowski, MD, Gregorio Delgado, MD, Robert W. Holloway, MD, Michael Farrell, PhD, A. Bennett Jenson, MD, and Wayne D. Lancaster, PhD
Washington, D.C.

Human papillomavirus type 16 deoxyribonucleic acid is shown within tumor metastases from cervical cancer primary lesions with in situ molecular hybridization.

Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy 1337
Miguel Isla, DVM, PhD, and Donald C. Dyer, PhD
Ames, Iowa

The vasodilating effects of nifedipine, D-600, and sodium nitroprusside in uterine arteries from pregnancy are analyzed, and their possible use for preeclampsia treatment is discussed.

Fetal and maternal response to intravenous infusion of a thromboxane synthetase inhibitor 1345
John P. Cardin, Jr., MD, Michael G. Ross, MPH, MD, M. Gore Ervin, PhD, A. Victoria Schaffer, MD, Frank L. Douglas, PhD, MD, and John P. Simke
Torrance, California, and Summit, New Jersey

Administration of a thromboxane synthetase inhibitor to normotensive pregnant ewes resulted in maternal serum thromboxane B_2 suppression, unchanged maternal and fetal hemodynamics, and minimal placental drug transfer.

Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors 1350
S. Devaskar, MD, K. McMenamy, MD, L. Holtzclaw, BS, and F. Sadiq, MD
St. Louis, Missouri

Long-term exposure to high or low insulin concentrations alters the liver insulin receptor abundance but fails to affect brain insulin receptors in rat mothers and fetuses.

Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb 1357
René P. Paulick, MD, Rebecka L. Meyers, MD, Colin D. Rudolph, MD, PhD, and Abraham M. Rudolph, MD
San Francisco, California

Prostaglandins have the effect of maintaining a modest degree of relaxation of the ductus venosus and hepatic microcirculation during fetal development.

Contents

LETTERS TO THE EDITORS

Definition of fetal asphyxia Richard B. Clark, MD *Little Rock, Arkansas* 1364

Reply John W. C. Johnson, MD, *and Douglas S. Richards, MD Gainesville, Florida* 1364

Cocaine effects on fetal behavioral state Bannie L. Tabor, MD, and Alex Soffici, MD *Torrance, California* 1364

Reply Roderick F. Hume, Jr., MD, MAJ, MC, USA *Washington, D.C.* 1365

Preeclampsia—An endothelial cell disorder plus "something else"? W. A. Liston and D. C. Kilpatrick, BSc, PhD *Edinburgh, Scotland* 1365

Reply James M. Roberts, MD, and Robert N. Taylor, MD, PhD *San Francisco, California* 1366

Possible therapeutic applications of cordocentesis David M. Sherer, MD, and Jacques S. Abramowicz, MD *Rochester, New York* 1366

Serum levels of interleukin-2 and tumor necrosis factor-α in women with recurrent abortion Peter Mallmann, Andreas Werner, and Dieter Krebs *Bonn, West Germany* 1367

What is birth asphyxia? Richard B. Clark, MD, and J. Gerald Quirk, MD, PhD *Little Rock, Arkansas* 1367

Reply James A. Thorp, MD *Kansas City, Missouri* 1368

Embryo migration responsible for ectopic pregnancies Botros Rizk, MB, BCh, and Peter Brinsden, MB, BS *London, England* 1369

Responsibility expands to extended family members Kay L. Saving, MD, Rodney A. McLaren, MD, Tim C Miller, MD, and Mary O'Connor, MSSW *Peoria, Illinois* 1369

Does prophylactic amnioinfusion make a difference? Suneet P. Chauhan, MD *New York, New York* 1370

Reply Thomas H. Strong, Jr., MD, Gretchen Hetzler, MD, and Richard H. Paul, MD *Phoenix, Arizona, and Los Angeles, California* 1370

Cordocentesis and immune thrombocytopenia—Continued Carl P. Weiner, MD *Iowa City, Iowa* 1371

Maternal serum unconjugated estriol levels are lower in the presence of fetal Down syndrome James E. Haddow, MD, Glenn E. Palomaki, BS, George J. Knight, PhD, Jacob A. Canick, PhD, Nicholas J. Wald, and Howard S. Cuckle, DPhil *Scarborough, Maine, Providence, Rhode Island, and London, England* 1372

Reply James N. Macri, PhD, Ramana V. Kasturi, BE, MS, David A. Krantz, BS, Edward J. Cook, PhD, Shirazali G. Sunderji, MD, and John W. Larsen, Jr., MD *Carle Place and Syracuse, New York, and Washington, D.C.* 1373

Measures of test performance and predictive values Nicholas Kadar, MD *Philadelphia, Pennsylvania* 1374

Reply Eyal Schiff, MD, Orit Moran, MD, Gilad Ben-Baruch, MD, and Joseph Menczer, MD *Tel Hashomer, Israel* 1375

Toxicity of vancomycin given during pregnancy J. B. Gouyon, MD, and A. M. Petion, MD *Dijon, France* 1375

Reply Milagros P. Reyes, MD, and Enrique M. Ostrea, Jr., MD *Detroit, Michigan* 1376

Ethics of new reproductive technologies Robert J. Blaskiewicz, MD *St. Louis, Missouri* 1376

Reply Professor Sir Malcolm Macnaughton *Glasgow, Scotland* 1376

Twin transfusion syndrome Virginia J. Baldwin, MD *Vancouver, British Columbia, Canada* 1376

Reply Dinesh M. Shah, MD, and David Chaffin, MD *Nashville, Tennessee* 1377

(Contents continued on page 18A)

Contents continued from page 17A

ITEMS

Items 1378

CORRECTION

Fetal thoracic circumference: A prospective cross-sectional study with real-time ultrasound 1363
(Fong K, Ohlsson A, Zalev A. 1988;158:1154-60)

INFORMATION FOR AUTHORS

Information for authors 25A

Checklist and copyright statement 29A

Index to advertisers on page 65A

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

The JOURNAL is abstracted and/or indexed in *Index Medicus, Science Citation Index, Current Contents/Clinical Medicine, Current Contents/Life Sciences, Excerpta Medica, BRS Colleague,* and MEDLINE.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Mosby–Year Book, Inc., for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page per copy is paid directly to CCC, 27 Congress St., Salem, MA 01970; phone (508)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/90 $00.00 + .10.



"I was devastated when I found out I had genital herpes. What a relief when my doctor recommended a treatment program including Zovirax Ointment."

Zovirax® (acyclovir) Ointment 5% is an ideal complement to any treatment program for initial herpes genitalis. As clinical studies confirm, Zovirax Ointment 5% significantly lessens the duration of pain, shortens viral shedding, *and* speeds healing.[1-3]

Whenever the comfort of a topical antiviral medication is desired, prescribe Zovirax Ointment 5% for initial herpes genitalis. It suits her needs—just when she needs it most.

In initial herpes genitalis,

ZOVIRAX®
(ACYCLOVIR)
OINTMENT 5%
Antiviral Action... Proven Results

Please see brief summary of prescribing information on next page.

References: **1.** Lacey CJN, Meaden JD, Darville JM, et al. Topical acyclovir ointment in the treatment of genital herpes simplex virus infections. *Eur J Sex Trans Dis.* 1985;2:167-170. **2.** Thin RN, Nabarro JM, Parker JD, Fiddian AP. Topical acyclovir in the treatment of initial genital herpes. *Br J Vener Dis.* 1983;59:116-119. **3.** Corey L, Benedetti JK, Critchlow CW, et al. Double-blind controlled trial of topical acyclovir in genital herpes simplex virus infections. Acyclovir symposium. *Am J Med.* 1982;73:326-334.

ZOVIRAX®
(ACYCLOVIR)
OINTMENT 5%

Before prescribing, please consult full package insert, a summary of which follows:

INDICATIONS AND USAGE: Zovirax (Acyclovir) Ointment 5% is indicated in the management of initial herpes genitalis and in limited nonlife-threatening mucocutaneous Herpes simplex virus infections in immunocompromised patients. In clinical trials of initial herpes genitalis, Zovirax Ointment 5% has shown a decrease in healing time and in some cases a decrease in duration of viral shedding and duration of pain. In studies in immunocompromised patients with mainly herpes labialis, there was a decrease in duration of viral shedding and a slight decrease in duration of pain.

By contrast, in studies of recurrent herpes genitalis and of herpes labialis in nonimmunocompromised patients, there was no evidence of clinical benefit; there was some decrease in duration of viral shedding.

Diagnosis: Whereas cutaneous lesions associated with Herpes simplex infections are often characteristic, the finding of multinucleated giant cells in smears prepared from lesion exudate or scrapings may assist in the diagnosis.[1] Positive cultures for Herpes simplex virus offer a reliable means for confirmation of the diagnosis. In genital herpes, appropriate examinations should be performed to rule out other sexually transmitted diseases.

CONTRAINDICATIONS: Zovirax Ointment 5% is contraindicated for patients who develop hypersensitivity or chemical intolerance to the components of the formulation.

WARNINGS: Zovirax Ointment 5% is intended for cutaneous use only and should not be used in the eye.

PRECAUTIONS:

General: The recommended dosage, frequency of applications, and length of treatment should not be exceeded (see DOSAGE AND ADMINISTRATION). There exist no data which demonstrate that the use of Zovirax Ointment 5% will either prevent transmission of infection to other persons or prevent recurrent infections when applied in the absence of signs and symptoms. Zovirax Ointment 5% should not be used for the prevention of recurrent HSV infections. Although clinically significant viral resistance associated with the use of Zovirax Ointment 5% has not been observed, this possibility exists.

Drug Interactions: Clinical experience has identified no interactions resulting from topical or systemic administration of other drugs concomitantly with Zovirax Ointment 5%.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Acyclovir was tested in lifetime bioassays in rats and mice at single daily doses of 50, 150 and 450 mg/kg/day given by gavage. These studies showed no statistically significant difference in the incidence of benign and malignant tumors produced in drug-treated as compared to control animals, nor did acyclovir induce the occurrence of tumors earlier in drug-treated animals as compared to controls. In 2 *in vitro* cell transformation assays, used to provide preliminary assessment of potential oncogenicity in advance of these more definitive lifetime bioassays in rodents, conflicting results were obtained. Acyclovir was positive at the highest dose used in one system and the resulting morphologically transformed cells formed tumors when inoculated into immunosuppressed, syngeneic, weanling mice. Acyclovir was negative in another transformation system.

No chromosome damage was observed at maximum tolerated parenteral doses of 100 mg/kg acyclovir in rats or Chinese hamsters; higher doses of 500 and 1000 mg/kg were clastogenic in Chinese hamsters. In addition, no activity was found in a dominant lethal study in mice. In 9 of 11 microbial and mammalian cell assays, no evidence of mutagenicity was observed. In 2 mammalian cell assays (human lymphocytes and L5178Y mouse lymphoma cells *in vitro*), positive response for mutagenicity and chromosomal damage occurred, but only at concentrations at least 1000 times the plasma levels achieved in man following topical application.

Acyclovir does not impair fertility or reproduction in mice at oral doses up to 450 mg/kg/day or in rats at subcutaneous doses up to 25 mg/kg/day. In rabbits given a high dose of acyclovir (50 mg/kg/day, s.c.), there was a statistically significant decrease in implantation efficiency.

Pregnancy: *Teratogenic Effects*. Pregnancy Category C. Acyclovir was not teratogenic in the mouse (450 mg/kg/day, p.o.), rabbit (50 mg/kg/day, s.c. and i.v.) or in standard tests in the rat (50 mg/kg/day, s.c.). In a non-standard test in rats, fetal abnormalities, such as head and tail anomalies, were observed following subcutaneous administration of acyclovir at very high doses associated with toxicity to the maternal rat. The clinical relevance of these findings is uncertain.[2] There are no adequate and well-controlled studies in pregnant women. Acyclovir should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Zovirax is administered to a nursing woman.

ADVERSE REACTIONS: Because ulcerated genital lesions are characteristically tender and sensitive to any contact or manipulation, patients may experience discomfort upon application of ointment. In the controlled clinical trials, mild pain (including transient burning and stinging) was reported by 103 (28.3%) of 364 patients treated with acyclovir and by 115 (31.1%) of 370 patients treated with placebo; treatment was discontinued in 2 of these patients. Other local reactions among acyclovir-treated patients included pruritus in 15 (4.1%), rash in 1 (0.3%) and vulvitis in 1 (0.3%). Among the placebo-treated patients, pruritus was reported by 17 (4.6%) and rash by 1 (0.3%).

In all studies, there was no significant difference between the drug and placebo group in the rate or type of reported adverse reactions nor were there any differences in abnormal clinical laboratory findings.

OVERDOSAGE: Overdosage by topical application of Zovirax Ointment 5% is unlikely because of limited transcutaneous absorption (see Clinical Pharmacology).

DOSAGE AND ADMINISTRATION: Apply sufficient quantity to adequately cover all lesions every 3 hours 6 times per day for 7 days. The dose size per application will vary depending upon the total lesion area but should approximate a one-half inch ribbon of ointment per 4 square inches of surface area. A finger cot or rubber glove should be used when applying Zovirax to prevent autoinoculation of other body sites and transmission of infection to other persons. **Therapy should be initiated as early as possible following onset of signs and symptoms.**

HOW SUPPLIED: Zovirax Ointment 5% is supplied in 15 g tubes (NDC 0081-0993-94) and 3 g tubes (NDC 0081-0993-41). Each gram contains 50 mg acyclovir in a polyethylene glycol base. Store at 15°-25°C (59°-77°F) in a dry place.

REFERENCES

1. Naib ZM et al. *Cancer Res* 33:1452-1463, 1973.
2. Stahlmann R, Klug S, Lewandowski C, et al. *Infection:* 15(4): 261-262, 1987.

U.S. Patent No. 4199574

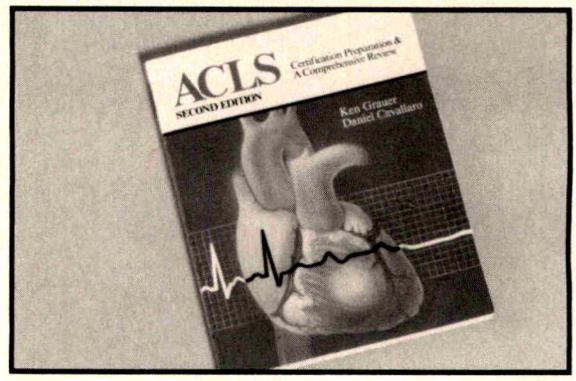
IMPROVING LIVES THROUGH ANTIVIRAL RESEARCH

Burroughs Wellcome Co.,
Research Triangle Park,
North Carolina 27709

Copr. © 1989 Burroughs Wellcome Co. All rights reserved.

ZO-1029

2nd Edition!



ACLS: Certification Preparation and A Comprehensive Review

By Ken Grauer, M.D., F.A.A.F.P. and Daniel Cavallaro, N.R.E.M.T.

"...I defy a reader to find so much useful information, together, in a easy-to-find place again!" (JEMS, review)

NEW sections on airway management, achieving intravenous access, pediatrics and medicolegal issues

NEW chapters on sudden cardiac death and myocardial infarction

1987. 480 pages, 477 illustrations. (Book Code: 01751) **$23.95** (U.S.); $31.25 (Can.)

Save time! Call toll-free: 800-426-4545, Monday-Friday, 7AM-7PM, Central Time FAX orders, 314-432-1380 Attn: Direct Marketing Please mention this number when calling: **FLR-618-999-03** In Canada, call collect: 416-298-2465 For Customer Service: 800-633-6699 Monday-Friday, 7AM-6PM, Central Time

Mosby-Year Book, 11830 Westline Industrial Drive, St. Louis, MO 63146 **Mosby-Year Book, Ltd.,**5240 Finch Avenue East, Scarborough, Ontario, Canada M1S 5A2

YES! Please send ____ copies of **(01751) ACLS:** CERTIFICATION PREPARATION AND A COMPREHENSIVE REVIEW, priced at **$23.95** (U.S.); $31.25 (Can.), on no-risk, 30-day approval.

SAVE MONEY! Send a check with your order or charge it to your credit card. Mosby-Year Book pays the postage on all prepaid orders. Please add $3.50 per order to cover handling charges. Other orders will be billed for postage and handling.

☐ Payment enclosed ☐ Bill me, plus postage and handling

Charge my: ☐ MasterCard ☐ VISA ☐ AmEx

Card # _____ Exp. date _____

Signature _____

Business phone (_____) _____

Name _____

Address _____

City _____

State _____ Zip _____

If using a purchase order, please attach this coupon and send to Pat Newman at the Mosby-Year Book address. Outside the U.S. and Canada, please pay by check or money order in dollar equivalent. 30-day approval good in U.S. and Canada. **Prices subject to change without notice. Add applicable sales tax.**

Mosby Year Book

FLR-618-999-03

American Journal of Obstetrics and Gynecology

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*
FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*
E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*
STEVEN G. GABBE, *Associate Editor*
THOMAS J. GARITE, *Associate Editor*
MOON H. KIM, *Associate Editor*
ALBERTO MANETTA, *Associate Editor*

Advisory committee on policy

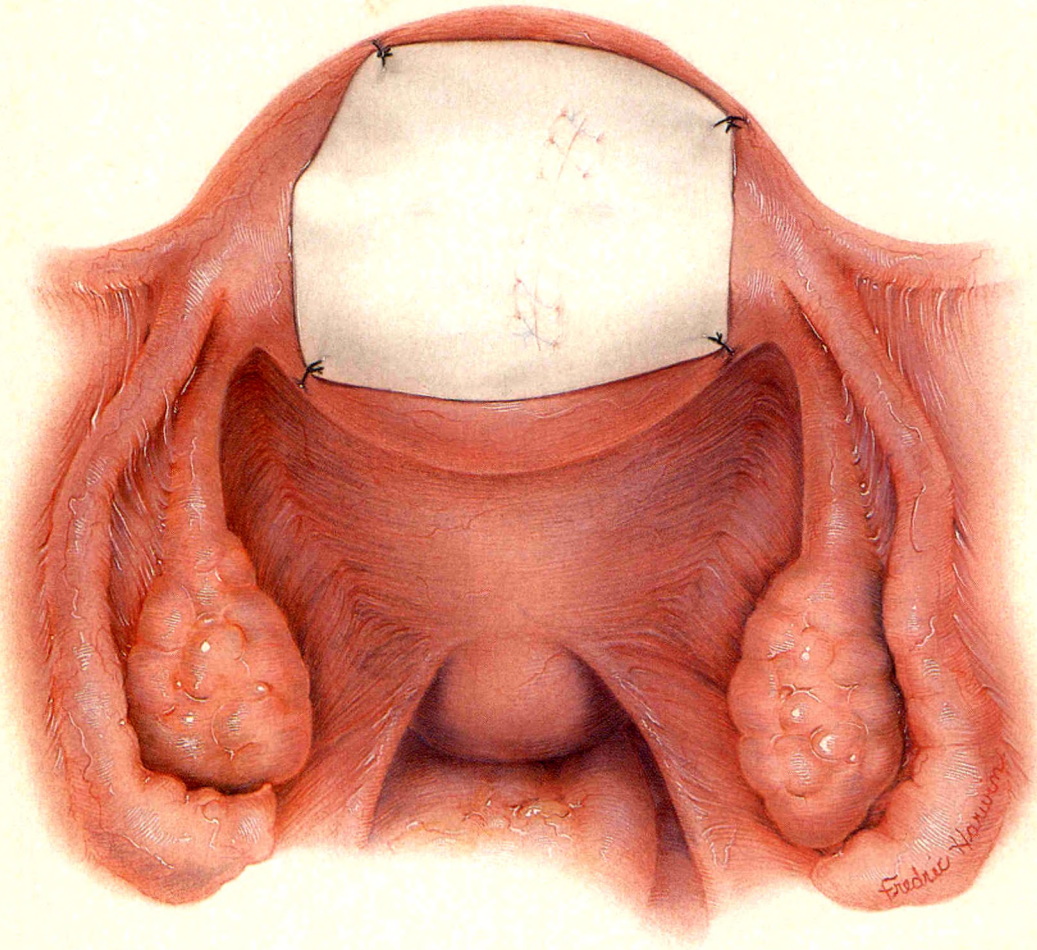
Garland Anderson
William R. Anderson
Mason C. Andrews
E. Forrest Boyd
Robert C. Cefalo
Donald R. Coustan
A. Jerome Freeland
Norman F. Gant
W. Glenn Hurt
Walter S. Keifer
M. Tucker Laffitte, Jr.
Emmet J. Lamb
Roger A. Lobo
James M. Maharry
L. Russell Malinak
Douglas J. Marchant
Arthur M. McCausland
James A. Merrill
Frank Miller
Daniel R. Mishell, Jr.
John C. Morrison
Karl C. Podratz
Ruth W. Schwartz
James R. Scott
Henry A. Thiede
Carol Trumbold
Ronald V. Wade

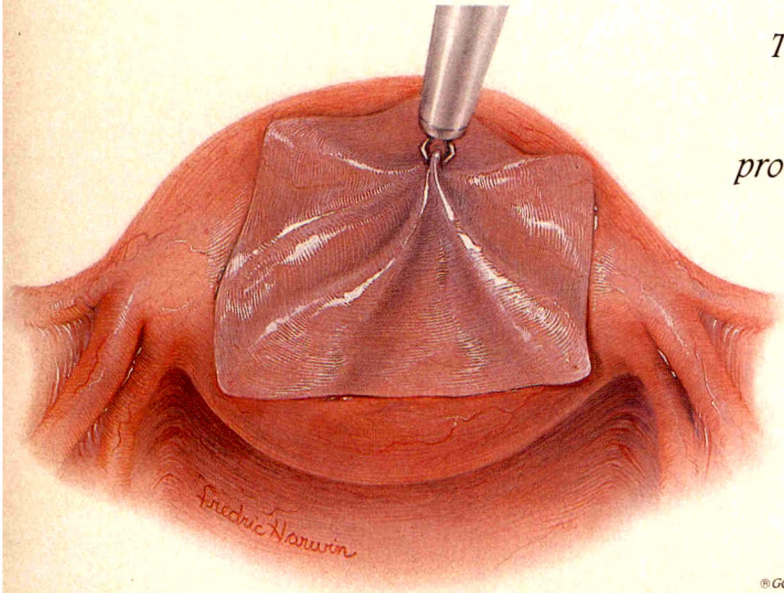
Board of corresponding editors

Luigi Carenza, *Rome, Italy*
T. K. A. B. Eskes, *Nijmegen, The Netherlands*
Wolfgang Künzel, *Giessen, West Germany*
Matsuto Mochizuki, *Kobe, Japan*
M. Reuben Peyser, *Tel Aviv, Israel*
Nils-Otto Sjöberg, *Malmö, Sweden*
Gordon M. Stirrat, *Bristol, England*
Claude Sureau, *Paris, France*
Carl Wood, *Melbourne, Victoria, Australia*
Olavi Ylikorkala, *Helsinki, Finland*

Publisher
MOSBY–YEAR BOOK, INC., 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*

Invariant Performance
Minimizing



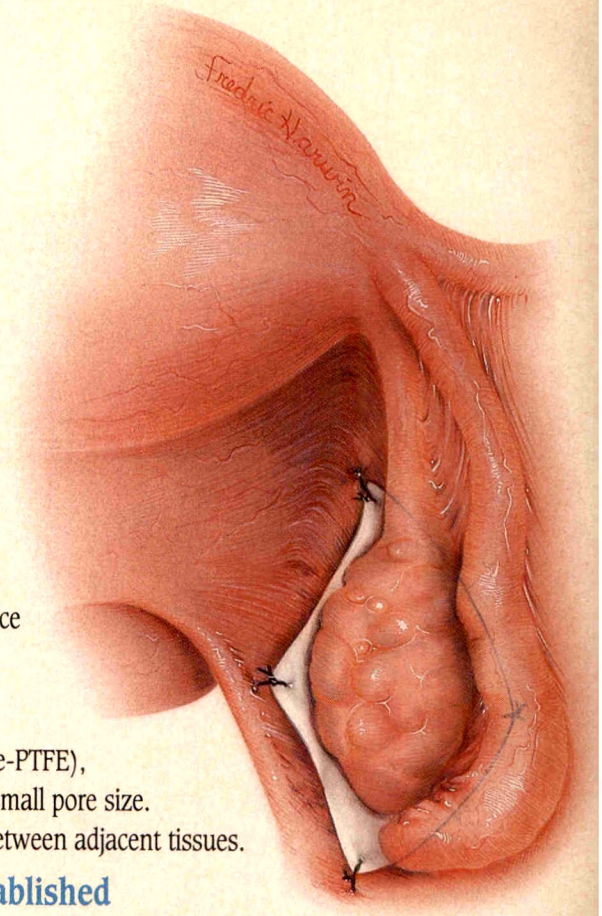


The GORE-TEX Surgical Membrane –
Configured for
Peritoneal Reconstruction
protects denuded areas from re-adhesion
while healing progresses beneath
the material.

®GORE-TEX is a registered trademark of W.L. Gore & Associates, Inc. ©1990 W.L. Gore & Associates, Inc.

P24,292

in Adhesions...



...The GORE-TEX® Surgical Membrane
The Choice in Peritoneal Reconstruction

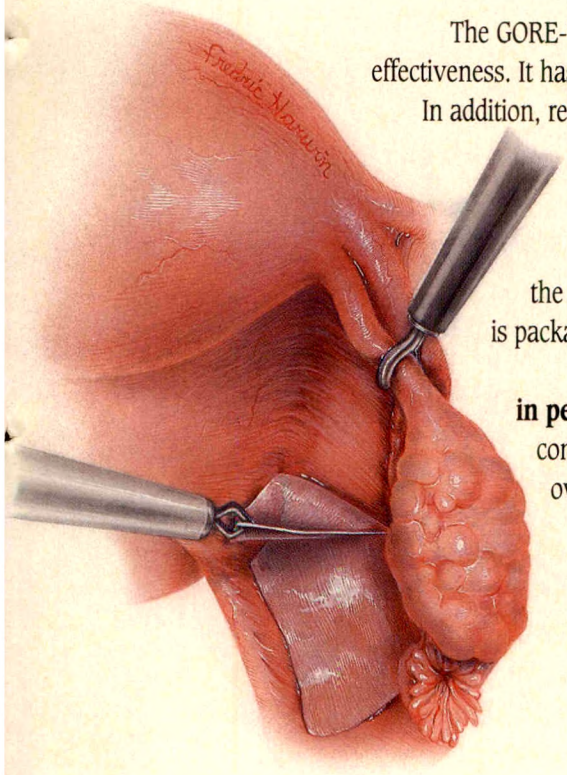
Successful peritoneal reconstruction requires dependable measures to minimize adhesion reformation following adhesiolysis. The invariant performance of the GORE-TEX Surgical Membrane aids in producing consistent results.

Performance by Design

Composed of the inert biomaterial, expanded polytetrafluoroethylene (e-PTFE), the GORE-TEX Surgical Membrane limits tissue attachment with an extremely small pore size. The nonabsorbable material is a physical barrier which minimizes adhesion between adjacent tissues.

Proven and Established

The GORE-TEX Surgical Membrane has a long record of safety and effectiveness. It has demonstrated reliability among cardiac surgeons for more than 14 years. In addition, recent use in a multi-center clinical study has substantiated safety and shown invariant performance in peritoneal repair.



Convenient

Available in specific configurations tailored for pelvic adhesion reduction, the GORE-TEX Surgical Membrane – *Configured for Peritoneal Reconstruction* is packaged sterile and **can be resterilized**.

Choose the GORE-TEX Surgical Membrane for invariant performance in peritoneal reconstruction. To receive further information or to consult an application specialist, call **800-437-8181**. For orders and overnight delivery 24 hours a day, please call **800-528-8763**.

GORE
Creative Technologies Worldwide



☐ *Please send further information on the GORE-TEX® Surgical Membrane.*
☐ *Please arrange for a free clinical sample.*
☐ *Please have your sales representative call for an appointment.*

Name _____

Address _____

City _____ State _____ Zip _____

Surgical Specialty _____

Hospital Affiliation _____

Telephone _____

W.L. GORE & ASSOCIATES, INC.
Woody Mountain Road, P.O. Box 900, Flagstaff, AZ 86002-0900

15259-10

Societies

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY

GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

CLEVELAND SOCIETY OF OBSTETRICIANS AND GYNECOLOGISTS

American Journal of Obstetrics and Gynecology

Copyright © 1990 by Mosby–Year Book, Inc.

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
Steven G. Gabbe, *Associate Editor*
Moon H. Kim, *Associate Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
Thomas G. Garite, *Associate Editor*
Alberto Manetta, *Associate Editor*
University of California, Irvine, Medical Center,
Department of Obstetrics and Gynecology, 101 The
City Drive, South, Building 26, Orange, CA 92668

Sherry A. Merryman, *Editorial Assistant*
Tel.: (714) 634-6572

Publisher
MOSBY–YEAR BOOK, INC., 11830 Westline
Industrial Drive, St. Louis, Missouri 63146-3318

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370 (ext. 7591)

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. Donald R. Coustan, Department of Obstetrics and Gynecology, Women and Infants Hospital of Rhode Island, 101 Dudley St., Providence, RI 02905.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. The specified copyright statement that follows the Information for Authors in each issue of the JOURNAL must be completed, signed by all authors, and submitted with the manuscript. (The Checklist and Copyright Statement may be torn from the JOURNAL or photocopied for submission.) If not completed in full, it will be returned to the author for completion.

Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the

Editors that manuscripts emanating from a particular institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed. The species of nonhuman animals used in an investigation must be named in the title, abstract, and key words of the manuscript.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

Disclaimer. Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name,* and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

For *black-and-white illustrations* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy photographic prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black-and-white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted.

Do not use paper clips or mar the surface of prints in any way.

Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with): author(s) name(s), title of article, number, and top marked clearly.

Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in

sequence after the references. Titles should be included in the legend, *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Letters to the Editors. Letters to the Editors must be submitted in duplicate, typed double-spaced, and the text of the manuscript must be limited to a maximum of 400 words [excluding references, name(s) and address(es) of the signer(s), and the phrase "To the Editors"]. The submitted Letter to the Editors must be accompanied by a signed copyright statement. If there are two or more signers of the Letter to the Editors, one of the authors may sign the copyright statement but must add to the statement the following: "I sign for and accept responsibility for releasing this letter on behalf of any and all co-signers." These requirements apply to all types of Letters to the Editors. Letters should be sent to Dr. Zuspan.

Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints

Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

CHECKLIST AND COPYRIGHT STATEMENT

Both the checklist and the copyright statement forms **must be completed** and sent with each submitted manuscript. If **not** completed, these forms will be returned to the author for completion.

These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

—The original and two copies of the manuscript are submitted.
—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.
—Three sets of unmounted glossy photographic prints are submitted, properly numbered and labeled on the back of each print.
—Three sets of computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back are submitted. None are dot matrix or photographic prints.
—Human experimentation has been approved by the local institution as stated in the Methods section.
—Guidelines for the care and use of animals approved by the institution have been followed as indicated in the Methods section.
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
—All pages are numbered in the following order: title page, condensation, abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
—Signed, written permission from both the copyright holder and the original author for the use of tables, figures, or quotations previously published and their complete references are enclosed with the manuscript.
—Signed, written permission for the use of quotations of personal communications and unpublished data has been obtained from the person(s) being quoted and is enclosed.

Authorship

—In the covering letter that accompanies the submitted manuscript I/we have confirmed that all authors fulfilled both conditions required for authorship.

Conflict of interest

—In the covering letter that accompanies the submitted manuscript the commercial association of the author or of any co-authors that might pose a conflict of interest is described.

Previous publications

—Enclosed with the submitted manuscript are two reprints each of articles the author or co-authors have previously published or have in manuscript form dealing with same patients, same animals, same laboratory experiment, or same data, in part or in full, as those reported in the submitted manuscript. Further explanation is provided in the covering letter that accompanies the submitted manuscript.

Title page

—These elements are given in the following sequence, and are typed double-spaced.
—Title
—Author(s) name(s) and highest academic degree(s)
—City(ies), state(s), and country other than U.S. in which the study was conducted are given
—The name(s) of the institution(s) section(s), division(s), department(s) in which the study was performed is provided and the author(s) institutional affiliation at the time of the study is indicated
—Acknowledgment of financial support is cited
—Name, address, and business and home telephone numbers of author to whom requests for reprints are to be sent
—Reprints will not be available. This has been stated on the title page
—Since the corresponding author is different from the author to whom reprint requests are to be sent, his/her name, address, and business and home telephone numbers have been added
—At the bottom of the page, a running head not exceeding 52 characters (including word spaces) has been typed

Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract

—The abstract is typed double-spaced with required margins (on page 3) headed by the title and the author(s) name, not exceeding 150 words. For case reports and brief communications the limit of 50 words is not exceeded.
—Three to five key words or short phrases are placed beneath the abstract on page 3.

Tables

—Each table headed by a caption and numbered in Roman numerals is typed double-spaced on a separate page.
—Tables are cited in sequence in the text.

Figures

—Are numbered in Arabic numerals and are cited in numerical sequence in the text.

—Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

References

—Are typed double-spaced.

—Are numbered consecutively in the order they are cited in the text.

—The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors were followed.

—Abstracts, personal communications, and unpublished work are not used as numbered references but are mentioned in the text with the written approval of the person being quoted and the signed approval is enclosed.

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

COPYRIGHT STATEMENT

Must be signed by ALL authors

"The undersigned author(s) transfers all copyright ownership of the manuscript entitled

to Mosby–Year Book, Inc., in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author."

The responsible author(s) must be named_____ _____
(Type name) Signature

Each author's name must be typed underneath the signature.

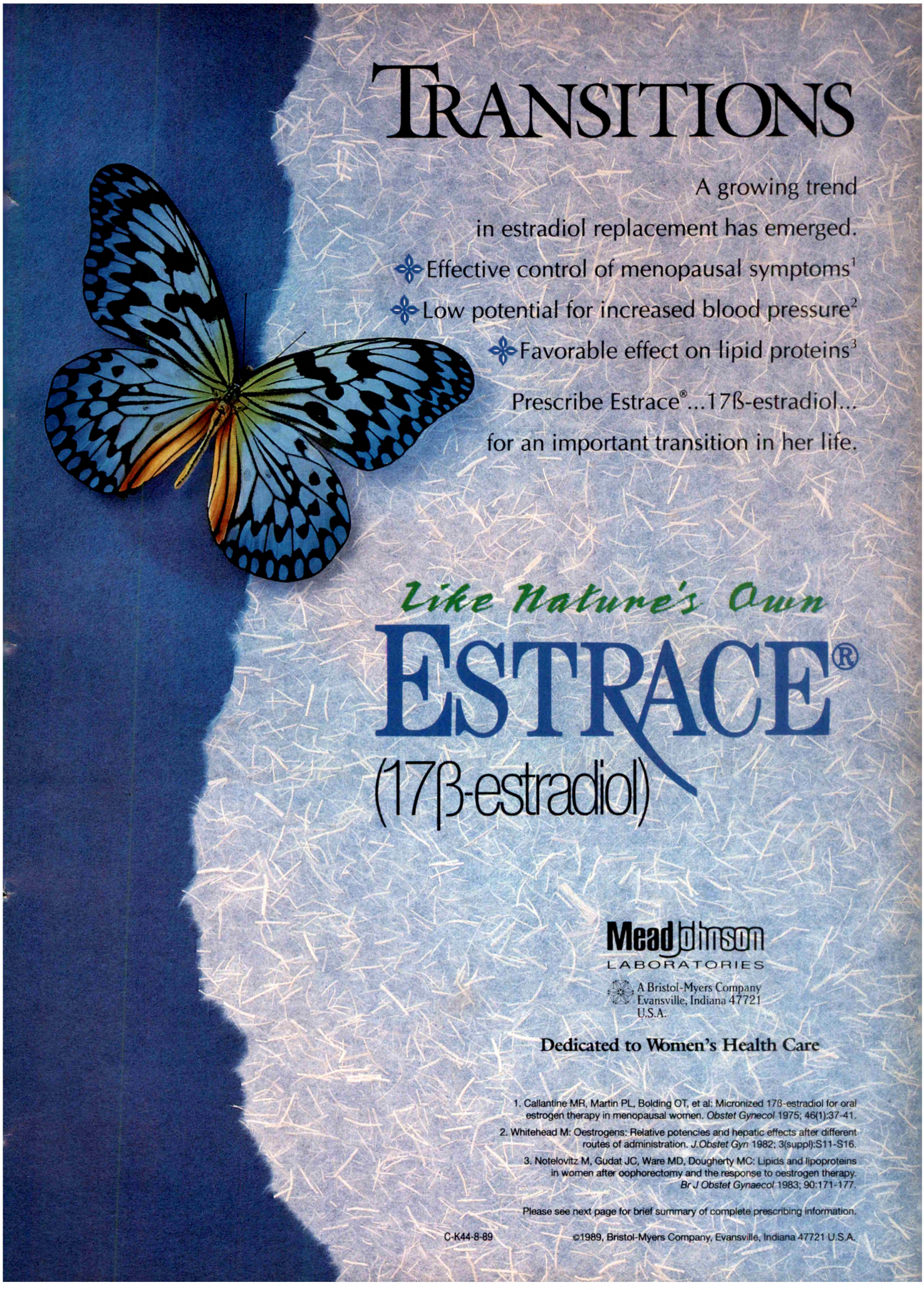
_____ _____

_____ _____

_____ _____

_____ _____

Date: _____


TRANSITIONS
A growing trend
in estradiol replacement has emerged.
Effective control of menopausal symptoms[1]
Low potential for increased blood pressure[2]
Favorable effect on lipid proteins[3]
Prescribe Estrace®...17ß-estradiol...
for an important transition in her life.
Like Nature's Own
ESTRACE®
(17β-estradiol)
Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.
Dedicated to Women's Health Care
1. Callantine MR, Martin PL, Bolding OT, et al: Micronized 17ß-estradiol for oral estrogen therapy in menopausal women. Obstet Gynecol 1975; 46(1):37-41.
2. Whitehead M: Oestrogens: Relative potencies and hepatic effects after different routes of administration. J.Obstet Gyn 1982; 3(suppl):S11-S16.
3. Notelovitz M, Gudat JC, Ware MD, Dougherty MC: Lipids and lipoproteins in women after oophorectomy and the response to oestrogen therapy. Br J Obstet Gynaecol 1983; 90:171-177.
Please see next page for brief summary of complete prescribing information.
C-K44-8-89
©1989, Bristol-Myers Company, Evansville, Indiana 47721 U.S.A.

ESTRACE® (17β-estradiol)

TABLETS, 1 mg and 2 mg VAGINAL CREAM, 0.01%

WARNING

1. **ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.** Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. **ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If Estrace is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

Indications: *Tablets:* Moderate to severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.); atrophic vaginitis; kraurosis vulvae; female hypogonadism; female castration; primary ovarian failure; breast cancer (for palliation only) in appropriately selected women and men with metastatic disease; prostatic carcinoma–palliative therapy of advanced disease. *Cream:* Estrace Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae. ESTRACE® HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Contraindications: *Estrogens should not be used in women (or men) with any of the following conditions:* Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: *Induction of malignant neoplasms.* Long term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning). At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. *Gallbladder disease.* A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement are more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used (except in treatment of malignancy) in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk. *Hepatic adenoma.* Hepatic adenomas appear to be associated with the use of oral contraceptives. Although rare, these may rupture and may cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General Precautions.* A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Fluid retention–Because estrogens may cause some degree of fluid retention, conditions which might be influenced by this factor such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen: increased sulfobromophthalein retention; increased prothrombin and factors VII and VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by volume, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnanediol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration. *Pregnancy Category X.* See Contraindications and Boxed Warning. *Nursing Mothers.* As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk. Estrace 2 mg tablets contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Adverse Reactions: (See Warnings regarding induction of neoplasia, adverse effects on the fetus, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives: *Genitourinary system*–Breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical eversion and in degree of cervical secretion; cystitis-like syndrome. *Breast*–tenderness, enlargement, secretion. *Gastrointestinal*–Nausea, vomiting; abdominal cramps, bloating, cholestatic jaundice. *Skin*–Chloasma or melasma which may persist when drug is discontinued; erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism. *Eyes*–Steepening of corneal curvature; intolerance to contact lenses. *CNS*–Headache, migraine, dizziness; mental depression; chorea. *Miscellaneous*–Increase or decrease in weight, reduced carbohydrate tolerance; aggravation of porphyria, edema, changes in libido.

Overdosage: Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea, and withdrawal bleeding may occur in females.

How Supplied: Estrace 1 mg; lavender scored tablets–*NDC* 0087-0755-01-Bottles of 100; *NDC* 0087-0755-44-Compacts of 25. Estrace 2 mg; turquoise scored tablets–*NDC* 0087-0756-01-Bottles of 100; *NDC* 0087-0756-44-Compacts of 25. Estrace Vaginal Cream; *NDC* 0087-0754-42 42.5 g Tube with one applicator calibrated for delivery of 1, 2, 3 or 4g.

Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.

C-K44-8-89

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 163 *number* 4 *part* 1 OCTOBER 1990

EDITORIAL

The first seventy years

The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY will celebrate its seventieth year in October 1990.

The lineage of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY comes from the *American Journal of Obstetrics and Diseases of Women and Children,* founded in 1869 and published successfully for 50 years, then succumbing to financial difficulties. The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY began from that journal a few months later, with a new publisher, The C. V. Mosby Company, now Mosby–Year Book, Inc. The name changed slightly but the previous editor, Dr. George Kosmak, continued and spent 33 additional years editing the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY. When the JOURNAL began in 1920, the intent was to publish original communications, research, and special society transactions.

There has been a continuity of editors during the past seventy years. As a matter of fact, there have been only eight editors during this period of time, and they are as follows:

George W. Kosmak	1920-1953	(33 years)
Hugo Ehrenfest	1935-1942	(7 years)
Howard C. Taylor, Jr.	1953-1969	(16 years)
William J. Dieckmann	1953-1964	(11 years)
Allan C. Barnes	1958-1971	(13 years)
John I. Brewer	1958-1990	(32 years)
Edward J. Quilligan	1971-present	(19 years)
Frederick P. Zuspan	1971-present	(19 years)

Also, many of the editors served as associate editors before their tenure as editor.

How appropriate it is today to appreciate the editorial from the *American Journal of Obstetrics and Diseases of Women and Children* published in 1869, which says the following:

> There has probably been no period in which Obstetrics and Gynecology have engaged more attention both at home and abroad than they do at the present time, and the tremendous strides which the above branches of the science of medicine have made within the last few years have necessarily given us a literature of the same, which equals, if it does not excel that of any other branch. It is to be regretted, however, that the practical part of our profession is so arduous, and the fulfillment of its duties consumes so much time, that weeks, and doubtless in many instances even months, elapse before we become familiar with the latest and accepted views and teachings of those who are recognized as authorities on their subject; a few moments, however, given to the perusal of a Journal will often supply the place of original works, by giving extracts, reviews, and a general synopsis of the same, while its original communications, when carefully selected, will likewise afford valuable and practical information.
>
> E. Noeggerath, MD, and B. F. Dawson, MD, Editors
> *American Journal of Obstetrics and Diseases of Women and Children,* 1869

The JOURNAL has changed and evolved during these past seventy years in an effort to constantly provide readers with current facts in the area of reproductive medicine. Our sections in the JOURNAL have been divided into Clinical and Basic Science areas. The present trend of investigation tends to be toward a narrow but more intense arena in the reproductive sciences, namely, those involved with maternal-fetal medicine, reproductive endocrinology-infertility, gynecologic oncology, and general obstetrics-gynecology. The Editors and Publisher believe that information and discoveries should be quickly and accurately communicated to the reader. During the past four years our publication time, from the date of acceptance, has been 4.7 months.

Last year we published 629 articles in twelve issues of the JOURNAL. Seventy-two percent of those articles originated in the United States, nine percent in Canada, and nineteen percent from abroad. This distribution has remained relatively constant for the last four years.

The majority of our articles are regular, nonsociety articles, but other categories include society articles, case reports, brief communications, and Current Investigation, Current Development, and Clinical Opinion sections. Letters to the Editors totaled 239, of which 202 were accepted.

Statistics, 1989

Category	*Processed*	*Accepted*	*Percent*
Regular nonsociety	1431	326	23
Society	217	197	91
Case reports and brief communications	245	86	35
Current Investigation	4	2	50
Current Development	8	6	76
Clinical Opinion	29	16	55

The distribution of articles is divided among Fetus-Placenta-Newborn 36%, Gynecology 25%, Obstetrics 38%, Ethics and Education 1%, Clinical or Clinical Investigation 83%, and Basic Science 17%.

Each regular article in the JOURNAL is approximated at 5 pages whereas case reports and brief communications are 2 pages.

A formal peer review process for all manuscripts was established many years ago. Society articles, as well as regular articles, all receive peer review. The consultants are qualified authorities in their respective fields, and without them the JOURNAL would not be of the quality that it is today. We extend a heart-filled thanks to all who have participated in this arduous and very important task of peer review.

Evolution of the JOURNAL has included a change from a monthly publication (for 42 years, from 1920 until 1962) to a semi-monthly publication. The editorial advisory board and others recommended that we go back to once-a-month publication in 1986. In 1985 we developed a new format separating Clinical and Basic Science articles but still maintained the three classifications of Obstetrics, Gynecology, and Fetus-Placenta-Newborn.

The Editors and Publisher are proud of the quality of this JOURNAL, but we also feel that the editorial advisory board and its peer consultants have played an instrumental role in the achievements of the JOURNAL to the heights it now enjoys. It is in the top two percent of more than 4000 journals published in the world today. The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY ranks first among obstetrics and gynecology journals in the *Science Citation Index.*

Beginning with the next volume another change will take place in the editorial makeup of the JOURNAL. Dr. John I. Brewer will become an emeritus editor, and Dr. Quilligan and Dr. Zuspan will assume the editor-in-chief role. Two associate editors, Dr. Steven Gabbe and Dr. Moon Kim, will assist Dr. Zuspan. Another two associate editors, Dr. Thomas Garite and Dr. Alberto Manetta, will assist Dr. Quilligan. This is a brief capsule of the first 70 years of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY. We thank everyone who has played a role in its success.

The Editors and Mosby–Year Book, Inc.

Clinical Opinion

Drug prophylaxis for human immunodeficiency virus–infected pregnant women: Ethical considerations

Howard L. Minkoff, MD,[a] and Jonathan D. Moreno, PhD[b]
Brooklyn, New York

More than 5000 women infected with human immunodeficiency virus give birth annually in the United States. Many of these women are offered human immunodeficiency virus tests in prenatal settings. One of the incentives for them to be tested is access to medications that have been shown to prolong disease-free intervals in nonpregnant human immunodeficiency virus–infected individuals. However, the use of those medications during pregnancy has not been well studied and some have cautioned against their prenatal use. Thus consideration of these agents may be deferred by some clinicians until the postpartum period. In this article we argue that the availability of such agents should be disclosed to women who are seropositive for human immunodeficiency virus as part of the informed consent process. (AM J OBSTET GYNECOL 1990;163:1111-4.)

Key words: Human immunodeficiency virus, pregnancy, pentamidine

Pregnancies complicated by human immunodeficiency virus (HIV) infections are becoming increasingly common in many parts of that United States. A number of health agencies have instituted anonymous surveillance programs that have provided estimates of the seroprevalence of HIV among pregnant women.[1-3] In New York State, for example, which has one of the most extensive programs, 0.66% of neonates are born with maternal HIV antibody in the blood.[1] Similar programs in Massachusetts have revealed that 0.75% of neonates in urban hospitals are HIV seropositive.[2] Thus it is clear that several thousand HIV-infected women give birth annually in the United States. Coincident with the recognition of this problem prenatal HIV counseling and testing programs have been recommended by a variety of agencies (e.g., Centers for Disease Control [CDC],[4] American College of Obstetricians and Gynecologists[5]) and have been instituted in many prenatal centers.[6, 7]

One of the incentives for women to undergo testing has been the widely publicized benefits of zidovidine for asymptomatic HIV-infected individuals with CD4 counts <500 mm^3. Zidovudine, along with agents used for *Pneumocystis carinii* pneumonia prophylaxis (e.g., aerosol pentamidine), holds the hope of prolonged disease-free intervals for infected individuals. Since a common site of testing has been prenatal clinics and since experience with these agents in pregnancy is lacking, obstetricians are not infrequently faced with the dilemma of choosing whether appropriate prenatal care of infected women involves the use of, or even discussions regarding the use of, these drugs.

Although these agents are reported to be life-extending, the CDC still states that the use of pentamidine prophylaxis in pregnancy, for example, "is inadvisable."[8] When some clinicians are confronted with this fairly strong caveat, it is probable that they will withdraw the drug from their patient's consideration until delivery has occurred. The appropriateness of that approach has not been formally considered. In this article we argue that the availability of HIV agents such as pentamidine should be disclosed to HIV-seropositive pregnant women as part of the informed consent process.

In general physicians caring for pregnant patients counsel them about the risks, benefits, and uncertainties of various therapeutic options. Patients are then free to pursue what they perceive to be the best interest of their fetuses and themselves. In the case of HIV disease this counseling includes discussions about the significance of HIV disease in pregnancy, the risks of perinatal transmission of HIV, and the availability of all reproductive options. What is less clear is whether

From the Departments of Obstetrics and Gynecology,[a] Pediatrics, Medicine, and the Division of Humanities in Medicine,[b] State University of New York Health Science Center at Brooklyn.
Reprint requests: Howard L. Minkoff, MD, Box 24, 450 Clarkson Ave., Brooklyn, NY 11203.
6/1/21552

a physician's perception of excessive fetal risk should ever override the mother's ability to gain access to medication and, if so, under what circumstances. Under circumstances in which the mother's life is threatened, physician's deference to fetal considerations protects the fetus at a price of imposing grave risk to the woman and constitutes a potentially dangerous assault on autonomy. Conversely, a physician's refusal to provide known teratogenic agents whose sole benefit would be cosmetic, would seem appropriate and ethical even if the patient was never informed of the agent's existence and potential benefits.

Newer agents used for HIV disease would not appear *a priori* to be at either of the cited extremes. Although the drugs can be life-extending, there is no evidence that deferring therapy with these agents for a few months would make a substantive difference in the natural history of the disease when CD4 counts start in the upper range at which prophylaxis is recommended (400 to 500 mm^3). The risks to the fetus are also unclear. Aerosol pentamidine achieves extremely low blood levels but may concentrate in tissues.[9] Zidovidine has not proved harmful in some lower animal models, has much greater affinity for ribonucleic acid than deoxyribonucleic acid,[10] but can have serious side effects in adults. Thus enough ambiguity about risks and benefits exists to warrant formal consideration of the proper framework for their use in pregnancy and for the proper repository of ultimate responsibility for instituting therapy.

To follow an application of informed consent doctrine to decision making in pregnancy, we turn to several specific issues concerning the counseling and treatment of fertile HIV-seropositive women. Because women with or at risk for HIV tend to be in an especially precarious social and financial position, we conclude by reiterating the importance of shared decision making as a model to guide physicians who work with these patients.

Informed consent and decision making in pregnancy

The doctrine of informed consent provides that physicians must disclose to competent adult patients all information about alternative therapies, including nontreatment, that would be material to an informed choice. This standard requires disclosure of all information that the patient cannot be reasonably expected to know. However, there is no definitive guidance for physicians concerning what degree or sorts of risk warrant nondisclosure. The courts have articulated two standards for disclosure of risks and alternatives: an "objective" test and a "subjective" test.[11] The objective test can be expressed as that which a reasonable, prudent person would decide under the circumstances, while the subjective test is that which an individual patient would consider a significant risk.

Pregnancy poses the additional complexity that another individual, the fetus, has weighty interests that are directly involved and must be taken into account. Certainly this is the case with regard to the fetus that is intended to be brought to term. Some will even consider the fetus virtually a patient in light of new technologies that can both test and treat some fetal conditions. When patients cannot speak for themselves, it is accepted that a surrogate decision maker should be identified. In spite of some highly publicized but eccentric court decisions,[12] it is customary to accept the mother as the appropriate representative of fetal interest, but her role in this process can be effective only to the extent that risks and alternatives are disclosed to her.

The objective test is clearly more restrictive than the subjective test in the sense that it requires that the construing of risk be "reasonable." Nevertheless, even with that standard the availability of HIV therapeutics such as pentamidine prophylaxis ethically should be disclosed to pregnant women with CD4 counts in the prescribed range. One who chose this therapy under these circumstances would not be making a choice that is clearly unreasonable or imprudent; although the life-extending benefits of the therapy might not be lost if it is deferred, there is no basis for certainty in that regard. Furthermore, risks presented to the fetus are also, at this time, highly uncertain.

By contrast, there are some therapeutic agents the selection of which during pregnancy would clearly be unreasonable and imprudent. An example is thalidomide, which presents a high teratogenic risk with the relatively slight benefit of increased maternal comfort. According to the objective test physicians are under no ethical obligation to disclose the availability of such agents.

Our conclusion is that the availability of pentamidine should be disclosed to pregnant women who are asymptomatic for HIV but whose CD4 counts would in principle warrant this prophylaxis. This position is consistent with the CDC's characterization of its use as "inadvisable." While a pregnant patient's decision to use the agent may not be the "best" decision in the sense that she should clearly be advised to do so, there is sufficient ambiguity to warrant respecting the patient's *prima facie* right to make an individual determination that is based on her own values. In other words, the question of whether a certain therapy is advisable must be distinguished from the question of whether its existence as an alternative should be disclosed. Thus it is of some concern that current CDC recommendations go on to state: "Prophylaxis can then be considered for use in the postpartum period."[8] The implication that

even "consideration" before delivery is ill-advised is not compatible with the collaborative process that should characterize clinical decision making.

The context for discussion of HIV agents with pregnant women

The disclosure and possible use of medications for the treatment of HIV in a pregnant woman will of course be closely bound up with a number of other decisions that will have to be made in the course of the pregnancy, including whether the woman should become pregnant, whether abortion is advisable should she become pregnant, and how decisions will be made for the child if he or she is born HIV-positive and if acquired immunodeficiency syndrome actually develops. Knowledge of the availability of aerosolized pentamidine and its potential risks could, for example, affect a decision to become pregnant or to continue a pregnancy. Similarly, knowledge of risks to the fetus could help impress on the mother the importance of identifying a surrogate decision maker for her future child should she be incapacitated.

The questions whether a women with or at risk for HIV should be counseled not to become pregnant or whether, once pregnant, she should be advised to terminate the pregnancy are beyond the scope of this article. However, it seems worth noting that there is nothing about HIV disease per se that requires the adoption of a position different from that in counseling any women with a life-threatening disease that poses some risk to a fetus. Health care providers are no less susceptible than anyone else to taking HIV as somehow *sui generis*.

Physician-patient relations

Underlying the particular question about the use of HIV therapeutics in pregnancy is a larger issue about relations between physicians and patients. The language of "disclosure" comports with the ancient Hippocratic tradition of secrecy and silence, one in which physicians hold the keys to sacred knowledge that in the hands of the uninitiated would present untold dangers. In an era when medical and surgical interventions harmed as often as they healed, there was some warrant for this attitude. But the growth of scientific medicine provides patients with more or less predictable options for their lives. Under these conditions there has been greater appreciation, at least rhetorically, that patients must be permitted to involve themselves as deeply as they wish in assessing and selecting their alternatives. The President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research characterized this process as one of "shared decision making."[13] Yet in both the clinics and the courts it remains more a goal than a reality.

The implementation of collaborative decision making requires that doctors engage in an open dialogue with their patients, which is a significant departure from the profession's tradition and is not a skill usually imparted in medical school.[14]

The familiar yet unique dilemma posed by the treatment of a pregnant patient is the possibility that maternal and fetal interests will conflict. The physician may feel the need to balance competing risks and benefits for two patients with few guidelines other than those derived from his or her own values. As long as the clinician's focus remains on the question of what should and should not be disclosed, his or her potentially conflicting roles as obstetrician and fetal advocate will persist. The treatment of HIV-infected women in pregnancy with unfamiliar medications should instead present an opportunity to work with patients to identify acceptable levels of risk. Physicians are under no moral obligation to pursue a course of treatment they regard as unconscionable, but in most instances they may find that an open discussion facilitates a result that is more acceptable than they had anticipated. At least the moral burden of the decision need not and should not rest entirely on the physician's shoulders.

The demography of HIV infections in pregnancy demands that physicians treating pregnant patients be particularly aware of this point. Currently almost 80% of HIV-infected infants have been born to black or Hispanic women, the vast majority of whom are poor and underinsured or uninsured. These patient characteristics, together with the clinical situations in which the patients are often seen, can combine to lessen the likelihood that they will be engaged by physicians in a joint endeavor to make decisions about their lives and that of their future children. Certainly there are instances in which providing a particular medical treatment may clearly be inadvisable. When ambiguity exists, however, informing a mother and empowering her to act on behalf of her fetus and herself "safeguards the autonomy of both parties."[14] Anything less denies the mother's rightful primacy both as fetal champion and as the focus of her physician's concern.

REFERENCES

1. Novick LF, Berns D, Stricof R, Stevens R, Ross K, Wethers J. HIV seroprevalence in newborns in New York State. JAMA 1989;261:1745-50.
2. Hoff R, Berardi VP, Weiblen BJ, Mahoney-Trout L, Mitchell ML, Grady GF. Seroprevalence of human immunodeficiency virus among childbearing women: estimating by testing samples of blood from newborns. N Engl J Med 1988;318:525-30.
3. Landesman S, Minkoff H, Holman S, McCalla S, Sinjin O. Serosurvey of human immunodeficiency virus infection in parturients. JAMA 1987;258:2701-3.
4. Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotrophic virus type

III/lymphadenopathy associated virus and acquired immunodeficiency syndrome. MMWR 1983;34:721-31.
5. American College of Obstetricians and Gynecologists. Prevention of Human immune deficiency syndrome. Washington DC: American College of Obstetricians and Gynecologists, 1987:1-4.
6. Lindsay MK, Peterson HB, Feng TI, Slade BA, Wallis S, Klein L. Routine antepartum human immunodeficiency virus infection screening in an inner city population. Obstet Gynecol 1989;74:289-94.
7. Minkoff HL, Landesman SH, Delke I, et al. Routinely offered prenatal HIV testing. N Engl J Med 1988; 319:1018.
8. Centers for Disease Control. Guidelines for prophylaxis against *Pneumocystis carinii* pneumonia for persons infected with human immunodeficiency virus. MMWR 1989;38:1-9.
9. Montgomery AB, Luce JM, Jurner J, et al. Aerosolized pentamidine as sole therapy for *Pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome. Lancet 1987;1:480-2.
10. Retrovirus product information 1987. Research Triangle Park, North Carolina: Burroughs Welcome, 1987.
11. Beauchamp T, Childress J. Principles of biomedical ethics 3rd ed. London: Oxford University Press, 1989: 88-91.
12. Annas G. Pregnant women as fetal containers. Hastings Cent Rep 1986;16:13-4.
13. President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research: Making health care decisions. Washington DC: US Government Printing Office, 1982:36.
14. Katz J. The silent world of doctor and patient. New York: Free Press, 1986:84.

Efficacy and safety considerations in women with uterine leiomyomas treated with gonadotropin-releasing hormone agonists: The estrogen threshold hypothesis

Andrew J. Friedman, MD, Susan M. Lobel, MD, Mitchell S. Rein, MD, and Robert L. Barbieri, MD

Boston, Massachusetts

Gonadotropin-releasing hormone agonists induce a reversible hypogonadotropic hypogonadal environment. Leiomyomas are common, estrogen-sensitive, benign neoplasms that decrease in size by 40% to 50% during gonadotropin-releasing hormone agonist treatment. During gonadotropin-releasing hormone agonist therapy most women are amenorrheic. After discontinuation of gonadotropin-releasing hormone agonist treatment, uterine and myoma size increase and a return to pretreatment menstrual patterns often occurs. Concerns about the safety of long-term hypoestrogenism have made long-term gonadotropin-releasing hormone agonist administration an undesirable treatment strategy. This article focuses on the use of gonadotropin-releasing hormone agonists as preoperative therapy in selected women undergoing hysterectomy or myomectomy and the combination of a gonadotropin-releasing hormone agonist with estrogen-progestin "add-back" treatment as a potential long-term medical therapy for women with symptomatic leiomyomas. Finally, an estrogen threshold hypothesis to assess the effects of circulating estrogen concentrations on different tissues, is presented. (Am J Obstet Gynecol 1990;163: 1114-9.)

Key words: GnRH agonist, uterine leiomyomas, hysterectomy, myomectomy, estrogen threshold hypothesis

The synthesis of the first gonadotropin-releasing hormone (GnRH) agonist in 1972[1] was followed by the development of a new class of drugs, the GnRH analogs, with potential widespread therapeutic applications in gynecology. Not since the development of oral contraceptives has there been so much excitement and enthusiasm among basic scientists, clinical investigators, and practitioners of reproductive medicine. It is anticipated that the GnRH agonists will have a major impact on gynecologic care in the 1990s.

Uterine leiomyomas are a common gynecologic problem that often present with symptoms related to enlarged uterine size, excessive bleeding, or both. Because

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School.
Reprint requests: Andrew J. Friedman, MD, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, 75 Francis St., ASB-1-3-073, Boston, MA 02115.
6/1/22419

these neoplasms are estrogen dependent, it follows that a GnRH agonist–induced hypogonadal state will lead to a reduction or resolution of symptoms related to excessive myoma size or bleeding. In 1983 Filicori et al.[2] were the first to report that treatment of a woman with a myoma with the GnRH agonist [D-Trp^6]-GnRH for 3 months was able to induce a reduction in fibroid volume by 77% and cause cessation of hypermenorrhea, thus leading to an improvement in hemoglobin concentration from 7.4 to 12.8 gm/dl. Although other pharmacologic agents such as danazol or progestins may successfully induce amenorrhea, neither has been demonstrated to consistently cause uterine and leiomyoma shrinkage. Unfortunately, most women experience a relatively rapid reversal of these therapeutic effects after cessation of GnRH agonist treatment.[3, 4] The transient nature of these beneficial effects has limited the efficacy of GnRH agonists as a primary medical therapy for uterine leiomyomas. In addition, the profound hypoestrogenic environment induced by GnRH agonists has raised concerns regarding the safety of long-term GnRH agonist treatment. This article reviews clinical investigations in which GnRH agonists have been used to treat uterine leiomyomas and proposes potential uses of these agents in common clinical situations. It is hoped that well-designed, controlled clinical trials will continue to be performed to clarify and add to the potential therapeutic applications of GnRH agonists in women with large or symptomatic leiomyomas.

Clinical trials with GnRH agonists

The majority of symptoms associated with fibroids are related to myoma size, excessive bleeding, or both. Numerous studies have demonstrated that treatment of women with uterine leiomyomas with GnRH agonists for 3 to 6 months consistently results in a reduction of uterine volume. The reported range of uterine shrinkage is 34% to 61% (average, 40% to 50%).[3-9] The decrease in individual fibroid volume is less predictable, probably because of the heterogeneity of myoma composition.[10] Most studies suggest that a 12-weeks period of GnRH agonist treatment is needed to achieve near maximal reduction in uterine size. Unfortunately, regrowth of the uterus and fibroids may occur rapidly, with the uterus often approaching pretreatment size 3 to 4 months after cessation of therapy.[3, 4] Because the effects of GnRH agonists on uterine size are transient, this treatment may be viewed as temporary or as a possible adjunct to surgical therapy.

Treatment with GnRH agonists also induces amenorrhea in approximately two thirds of treated women; one third experience hypomenorrhea or spotting.[4] The induction of an amenorrheic state may be dose dependent; larger doses of GnRH agonist suppress menses more reliably.

The safety and side effect profile of GnRH agonist treatment appears to be related to the induced hypoestrogenic state. Most women experience vasomotor flushes, with insomnia, headaches, mood changes, and vaginal dryness occurring less frequently.[3, 4] One consistent concern among investigators is the potential for the long-term administration of a GnRH agonist to lead to rapid bone resorption, analogous to the situation immediately after the menopause. The data on bone resorption are confusing, primarily because of differences in techniques used to assess changes in bone density. With precision of bone density measurement techniques that vary from 1% to 5%, small changes in bone densities are difficult to document with certainty. Most studies evaluating bone density have reported no decrease or reversible decreases in women treated with GnRH agonists for up to 6 months[11-13]; some studies suggest that the decrease in bone density during GnRH agonist treatment is only partially reversible.[14] There is no reported study that has evaluated changes in bone density in women treated with GnRH agonists for longer than 6 months.

Because use of GnRH agonists decreases uterine and fibroid size and induces amenorrhea, one therapeutic strategy might include preoperative treatment in some women undergoing hysterectomy or myomectomy. The use of a GnRH agonist as a preoperative adjunct is reviewed below.

Preoperative GnRH agonist treatment

Hysterectomy, the only definitive cure for uterine leiomyomas, is often recommended in women with large or symptomatic fibroids who have completed childbearing or when myomectomy is considered difficult or hazardous. Many experts believe that risks and complications (i.e., nonautologous blood transfusion, urologic injury) associated with hysterectomy in women with leiomyomas are strongly correlated with preoperative uterine size and the presence or absence of anemia.[15]

There is one published study that evaluated the efficacy of the preoperative use of a GnRH agonist in women with leiomyomas before hysterectomy.[6] Lumsden et al. treated 13 women with fibroids with goserelin depot 3.6 mg every 28 days for 3 months before operation. Fourteen untreated matched controls with leiomyomas also underwent hysterectomy. The group of women treated with goserelin had a mean reduction in uterine volume of 40% (351 to 210 cm^3). Mean intraoperative blood loss was significantly less in the treated group compared with controls (235 vs 350 ml, $p < 0.01$). The decrease observed in intraoperative blood loss in women pretreated with GnRH agonist may be a result of (1) decreased uterine size that afforded the surgeon more operating space to isolate, secure, and divide vascular pedicles and (2) decreased uterine

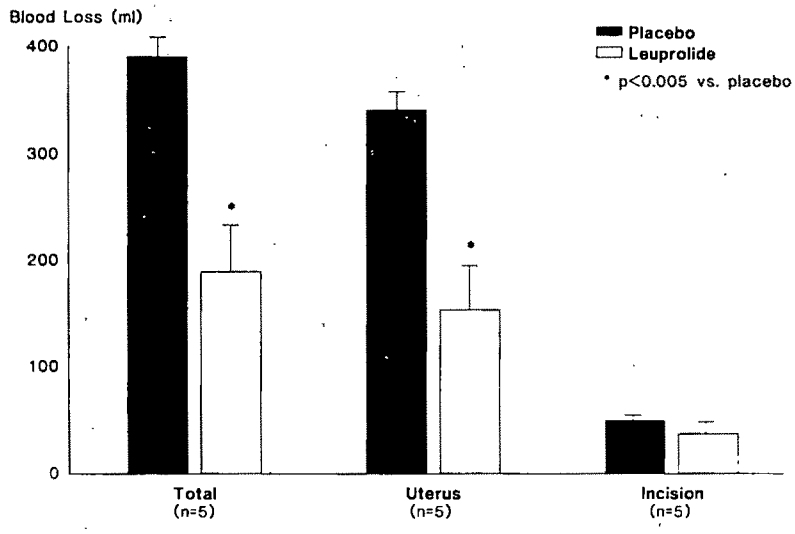


Fig. 1. Intraoperative blood loss at myomectomy for uteri with pretreatment volumes of ≥600 cm^3. Note: Total blood loss = uterine blood loss plus skin incision blood loss. (From Friedman AJ, Rein MS, Harrison-Atlas D, Garfield JM, Doubilet PM. A randomized, placebo-controlled, double-blind study evaluating leuprolide acetate depot treatment before myomectomy. Fertil Steril 1989;52:728. Reproduced with permission of the publisher, the American Fertility Society.)

artery blood flow, as demonstrated by Doppler studies,[16] suggesting that the uterus is less vascular after 2 to 4 months of GnRH agonist treatment.

In another study, 24 weeks of leuprolide acetate treatment in 7 women with uterine leiomyomas resulted in a significant increase in mean hemoglobin concentration from 12.7 to 14.1 gm/dl ($p < 0.01$).[7] Improvement in preoperative hematologic status may allow previously anemic women with hypermenorrhea the ability to donate blood for potential autologous blood transfusion. Because it is estimated that 30% of women with leiomyomas have menorrhagia or hypermenorrhea, GnRH agonist treatment may benefit a large number of women. The increase in preoperative hemoglobin concentration coupled with decreased intraoperative blood loss may decrease the need for nonautologous blood transfusion.

In addition to improving the patient's hematologic status, preoperative GnRH agonist treatment may offer other advantages. The decrease in uterine and myoma volume may "convert" an abdominal hysterectomy to one performed via a vaginal route. Vaginal hysterectomy has half the postoperative morbidity compared with abdominal hysterectomy.[17] In addition, fibroid shrinkage coupled with an atrophic endometrium may facilitate endoscopic resection of subserosal or submucous tumors. Finally, shrinkage of very large uteri may allow the surgeon to choose the stronger, more cosmetic Pfannenstiel skin incision instead of a midline vertical incision.

GnRH agonist treatment may also have a role in the preoperative management of women scheduled for myomectomy. Friedman et al.[9] reported that treatment with leuprolide acetate depot 3.75 mg every 4 weeks for 12 weeks before myomectomy resulted in less intraoperative blood loss in women with uterine leiomyomas at least 600 cm^3 in volume before treatment compared with placebo-treated controls. Mean intraoperative blood loss was 189 ml in five women with uteri > 600 cm^3 treated with leuprolide compared with 390 ml in five matched women treated with placebo ($p < 0.01$) (Fig. 1). When analysis was performed on all women (i.e., those with pretreatment volumes greater than and less than 600 cm^3) intraoperative blood loss was less in leuprolide-treated patients ($n = 9$) compared with controls ($n = 9$) (213 vs 302 ml) although this difference was not statistically significant. Not surprisingly, the difference in total intraoperative blood loss was attributed to differences in blood lost from uterine incisions and not from skin incisions. This study suggests that GnRH agonist treatment before myomectomy may help to decrease intraoperative blood loss in women with large uteri (i.e., >600 cm^3 or 16 gestational weeks). The potential advantages and disadvantages of preoperative GnRH agonist therapy are outlined in Table I.

GnRH agonist/hormone "add-back" studies

Several investigators have expressed concerns about primary GnRH agonist treatment in women with uterine leiomyomas including: (1) rapid regrowth of uterine-fibroid size after cessation of GnRH agonist therapy, (2) hypoestrogenic side effects, and (3) the potential for rapid or irreversible bone resorption in

women treated with GnRH agonists for more than 6 months. For these reasons several studies have evaluated the efficacy of GnRH agonist treatment combined with synthetic hormones. Administration of oral medroxyprogesterone acetate, 20 mg daily, has been shown to decrease the incidence of hot flushes in postmenopausal hypoestrogenic women.[18] In addition, this same dose of medroxyprogesterone acetate has been shown to decrease urinary calcium-creatinine and hydroxyproline/creatinine ratios in postmenopausal hypoestrogenic women.[19] Friedman et al.[7] evaluated the simultaneous combination treatment of leuprolide acetate, 0.5 mg subcutaneously, each day plus oral medroxyprogesterone acetate, 20 mg daily, in nine women with leiomyomas. Although eight of nine women treated reported no hot flushes, the mean decrease in uterine volume was only 14%. It is not certain why the addition of medroxyprogesterone acetate blocked the expected 40% to 50% decrease in uterine volume seen in women treated with leuprolide acetate alone.

In a small pilot study, five perimenopausal women aged 45 to 49 years with symptomatic leiomyomas were treated with daily leuprolide acetate 0.5 mg subcutaneously for 3 months, followed by leuprolide plus oral conjugated equine estrogens 0.625 mg days 1 through 25 each month and oral medroxyprogesterone acetate 10 mg days 16 through 25 each month for the ensuing 24 months.[20] The mean decrease in uterine volume was 49% at 3 months, with no further change in uterine volume over the next 2 years. All women had elimination of vasomotor flushes after conjugated equine estrogen-medroxyprogesterone acetate add-back; one woman required conjugated equine estrogen 1.25 mg on days 1 through 25. Bone density of the distal third of the radius (95% cortical bone, 5% trabecular bone) and ultradistal radius (25% cortical bone, 75% trabecular bone) demonstrated no changes over the 27-month treatment period as assessed by single-photon densitometry. Larger studies are needed to corroborate these findings before an add-back regimen can be recommended as a potential long-term medical treatment for fibroids.

The estrogen threshold hypothesis

This pilot study suggests that the concentration of estrogen required to reduce hot flashes and protect against bone loss may be less than the concentration of estradiol necessary to cause regrowth of myomas. This "estrogen threshold" hypothesis is shown schematically in Fig. 2. Three hypothetical estrogen zones are shown corresponding to different thresholds required for the growth or maintenance of various estrogen-dependent tissues. Zone A encompasses typical concentrations of estradiol seen throughout the menstrual cycle in premenopausal women. Estrogen concentrations in this range will support the growth or maintenance of estrogen-dependent tissues (i.e., myomas, bone). In zone C, estradiol levels are in the postmenopausal range, similar to estrogen concentrations after gonadal suppression with GnRH agonist treatment. Estrogen concentrations in this range cannot support tissue growth and, in fact, lead to regression of most estrogen-dependent tissues (i.e., myoma-myometrial shrinkage, bone resorption) and the presence of hypoestrogenic symptoms (i.e., hot flashes). In zone B estradiol levels are somewhat higher than castrate levels and are similar to those found in the early follicular phase. Estrogen concentrations in this range may be high enough to maintain the integrity of some estrogen-dependent tissues (i.e., bone) while causing regression in others (i.e., myomas). The goal of GnRH agonist hormone add-back therapy would be first to control the estrogen-dependent disease process by inducing a hypogonadal state with GnRH agonist treatment. Then, after hypoestrogenism has been achieved and the disease activity has been controlled, small amounts of estrogen, progestin, or both could be added back with the intent of reducing hot flashes and bone loss without causing reinitiation of myoma growth. With this strategy the clinician would attempt to maintain estrogen concentrations within zone B, the therapeutic window. The upper and lower estradiol concentrations in zone B would be expected to have considerable variation among individuals. Whereas some patients may have

Table I. Advantages and disadvantages of preoperative GnRH agonist treatment

Advantages gained by uterine-fibroid shrinkage
1. May allow vaginal hysterectomy
2. May decrease intraoperative blood loss
3. May allow Pfannenstiel incision
4. May facilitate endoscopic myomectomy
5. May decrease risk of ureteral injury
Advantages gained by induction of amenorrhea
1. May correct hypermenorrhea-menorrhagia–associated anemia
2. May improve ability to donate blood
3. May decrease need for nonautologous blood transfusion
4. May atrophy endometrium, facilitating hysteroscopic resection of submucosal tumors
Disadvantages
1. Delay to final tissue diagnosis
2. Degeneration of some leiomyomas necessitating piecemeal enucleation at myomectomy
3. Significant shrinkage of small intramural or submucosal tumors making them unrecognizable at myomectomy
4. Hypoestrogenic side effects (e.g., trabecular bone loss, vasomotor flushes, etc.)
5. Cost
6. Need to self-administer or receive injections in many cases
7. Vaginal hemorrhage in approximately 2% of patients

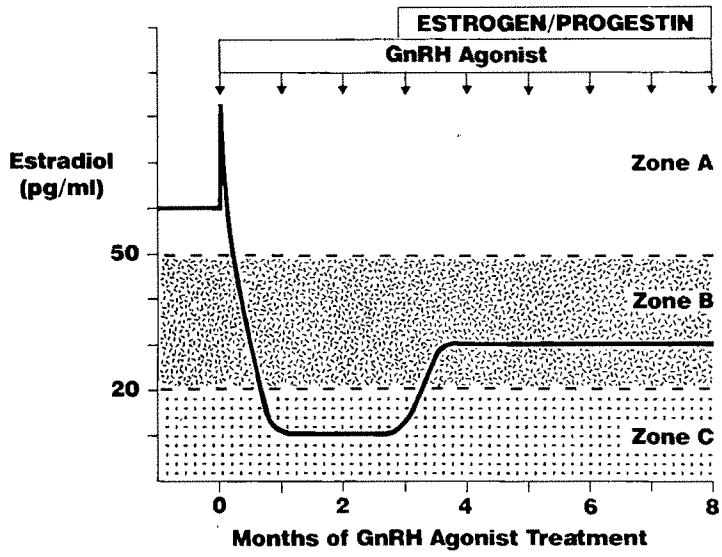


Fig. 2. Estrogen threshold hypothesis. *Zone A*, Typical circulating estradiol concentrations in premenopausal women are able to support myometrial-fibroid growth and prevent rapid bone resorption. *Zone B*, Therapeutic window. Myometrial and fibroid volume may decrease at these estradiol levels, but bone resorption is not accelerated. *Zone C*, Hypoestrogenism often attained during GnRH agonist therapy may lead to myometrial-fibroid shrinkage and rapid bone resorption. Note: Estradiol values for each zone may differ among patients.

Table II. Advantages and disadvantages of treating perimenopausal women with symptomatic leiomyomas with GnRH agonist

Advantages
1. Potential avoidance of operation
2. Reduction-resolution of symptoms
3. Possible correction of anemia in women with hypermenorrhea/menorrhagia
4. Possible facilitation of operation, if needed
Disadvantages
1. Delay-avoidance of tissue diagnosis
2. Inability to predict menopause necessitating several treatment courses, potentially increasing hypoestrogenism-related risks (i.e., osteoporosis)
3. Hypoestrogenic side effects
4. Cost
5. Need to self-administer or receive injections in many cases
6. Vaginal hemorrhage occurring in approximately 2% of patients

wide therapeutic windows making long-term medical management relatively easy to achieve, others may have narrow or absent therapeutic windows and would not be treated successfully by this GnRH-agonist hormone add-back approach.

Long-term GnRH agonist plus steroid add-back therapy will require extensive clinical investigation to clarify variables important to this therapeutic approach. For example, does the timing of estrogen and/or progestin add-back initiation affect treatment outcome? It is not known whether sequential therapy (i.e., GnRH agonist therapy for 3 to 6 months followed by GnRH agonist plus hormone add-back) is superior to simultaneous treatment (i.e., GnRH agonist and hormone add-back initiated simultaneously). The target estradiol concentration rate (i.e., the upper and lower limits of zone B) must be elucidated. Other variables that require further study include the optimum estrogen (i.e., estradiol, conjugated estrogens, estrone sulfate, ethinyl estradiol), and progestin (i.e., medroxyprogesterone acetate, norethindrone, norgestrel) formulations, the best route of administration (i.e., transdermal, oral, vaginal), length of therapy, need for calcium supplementation, and cost-effective monitoring of long-term treatment. Proper selection of patients is essential, and the physician must weight the potential benefits of long-term medical therapy against the potential risks of delaying or avoiding definitive tissue diagnosis.

Until further data are available we recommend that therapy for myomas be initiated with only GnRH agonist for 3 to 4 months and that initiation of steroid add-back be delayed until myoma-uterine volume has significantly decreased. As noted above, in one pilot study the simultaneous initiation of GnRH agonist therapy and progestin add-back resulted in no significant decrease in myoma volume.[7] By treating patients with a GnRH agonist alone for 3 to 4 months, the cells in the leiomyoma enter a quiescent state that may help to prevent a growth response to the steroid add-back.

If larger GnRH-agonist hormone add-back studies confirm the safety and efficacy of this treatment strat-

egy, it may be possible to treat perimenopausal women or other women with symptomatic leiomyomas who wish to delay or possibly avoid operation for longer than 6 months. The potential advantages and disadvantages of this treatment strategy are shown in Table II. GnRH-agonist hormone add-back regimens may also have applications in the long-term treatment of other sex-steroid-dependent disorders such as endometriosis, polycystic ovary syndrome, and premenstrual syndrome.

Treatment of women with uterine leiomyomas with GnRH agonists has been shown to result in a reduction in uterine and fibroid size, a decrease or abolition of menstrual flow with a resultant increase in hemoglobin concentrations, and a decrease in intraoperative blood loss in women undergoing hysterectomy or myomectomy. Long-term GnRH agonist treatment is limited by concerns about increased bone resorption and other hypoestrogenic side effects. Future research will focus on the safety and efficacy of long-term GnRH agonist hormone add-back regimens and preoperative treatment. In addition to the potential therapeutic uses of GnRH agonists in the treatment of uterine leiomyomas, these agents may be used as probes to help elucidate the biology of these common symptom-producing tumors.

We thank Ms. Carolyn Edwards for her expert preparation of this manuscript.

REFERENCES

1. Fujino J, Kobayashi S, Obayashi M, et al. Structure-activity relationships in the C-terminal part of luteinizing hormone-releasing hormone (LH-RH). Biohem Biophys Res Commun 1973;49:863.
2. Filicori M, Hall DA, Loughlin JS, Rivier J, Vale W, Crowley WF Jr. A conservative approach to the management of uterine leiomyoma: pituitary desensitization by a luteinizing hormone–releasing hormone analogue. Am J Obstet Gynecol 1983;147:726.
3. Friedman AJ, Barbieri RL, Benacerraf BR, Schiff I. Treatment of leiomyomata with intranasal or subcutaneous leuprolide, a gonadotropin-releasing hormone agonist. Fertil Steril 1987;48:560.
4. Friedman AJ, Harrison-Atlas D, Barbieri RL, Benacerraf B, Gleason R, Schiff I. A randomized, placebo-controlled, double-blind study evaluating the efficacy of leuprolide acetate depot in the treatment of uterine leiomyomata. Fertil Steril 1989;51:251.
5. Healy DL, Lawson SR, Abbott M, Baird DT, Fraser HM. Toward removing uterine fibroids without surgery: subcutaneous infusion of a luteinizing hormone-releasing hormone agonist commencing in the luteal phase. J Clin Endocrinol Metab 1986;63:619.
6. Lumsden MA, West CP, Baird DT. Goserelin therapy before surgery for uterine fibroids. Lancet 1987;1:36.
7. Friedman AJ, Barbieri RL, Doubilet PM, Fine C, Schiff I. A randomized, double-blind trial of gonadotropin-releasing hormone agonist (leuprolide) with or without medroxyprogesterone acetate in the treatment of leiomyomata uteri. Fertil Steril 1988;49:404.
8. Andreyko JL, Blummenfeld Z, Marshall LA, Monroe SE, Hricak H, Jaffe RB. Use of an agonistic analog of gonadotropin-releasing hormone (nafarelin) to treat leiomyomas: assessment by magnetic resonance imaging. Am J Obstet Gynecol 1988;158:903.
9. Friedman AJ, Rein MS, Harrison-Atlas D, Garfield JM, Doubilet PM. A randomized, placebo-controlled, double-blind study evaluating leuprolide acetate depot treatment before myomectomy. Fertil Steril 1989;52:728.
10. Maheux R, Guilloteau C, Lemay A, Bastide A, Fazekas ATA. Luteinizing hormone-releasing hormone agonist and uterine leiomyoma: a pilot study. Am J Obstet Gynecol 1985;152:1034.
11. Johansen JS, Riis BJ, Hassager C, et al. The effect of a gonadotropin-releasing hormone agonist analog (nafarelin) on bone metabolism. J Clin Endocrinol Metab 1988;67:701.
12. Matta WH, Shaw RW, Hesp R, Katz D. Hypogonadism induced by luteinizing hormone releasing hormone agonist analogues: effects on bone density in premenopausal women. Br Med J 1987;294:1523.
13. Tummon IA, Ali A, Pepping M, et al. Bone mineral density in women with endometriosis before and during ovarian suppression with gonadotropin-releasing hormone agonists or danazol. Fertil Steril 1988;49:792.
14. Dawood MY, Lewis V, Ramos J. Cortical and trabecular bone mineral content in women with endometriosis: effect of gonadotropin-releasing hormone agonist and danazol. Fertil Steril 1989;52:21.
15. Mintz PD, Sullivan MF. Preoperative crossmatch ordering and blood use in elective hysterectomy. Obstet Gynecol 1985;65:389.
16. Matta WHM, Stabile I, Shaw RW, Campbell S. Doppler assessment of uterine blood flow changes in patients with fibroids receiving the gonadotropin-releasing hormone agonist buserelin. Fertil Steril 1988;49:1083.
17. Dicker RC, Greenspan JR, Strauss LT, et al. Complications of abdominal and vaginal hysterectomy among women of reproductive age in the United States. Am J Obstet Gynecol 1982;144:841.
18. Schiff I, Tulchinsky D, Cramer D, Ryan KJ. Oral medroxyprogesterone in the treatment of postmenopausal symptoms. JAMA 1980;244:1443.
19. Mandel FP, Davidson BJ, Erlik Y, Judd HL, Meldrum DR. Effects of progestins on bone metabolism in postmenopausal women. J Reprod Med 1982;27:511.
20. Friedman AJ. Treatment of leiomyomata uteri with short-term leuprolide followed by leuprolide plus estrogen-progestin hormone replacement therapy for 2 years: a pilot study. Fertil Steril 1989;51:526.

Contraception and ectopic pregnancy risk

Adele L. Franks, MD,[a] Valerie Beral, MB, BS,[b] Willard Cates, Jr., MD, MPH,[b] and Carol J. R. Hogue, PhD[a]

Atlanta, Georgia

Studies of the association of ectopic pregnancy with contraception have generated a conflicting array of results because of methodologic differences between studies. We estimated the absolute incidence rates of ectopic pregnancy for various contraceptives by multiplying the pregnancy rate by the proportion of pregnancies with ectopic implantation for each method. Our results indicated a more than 500-fold difference in ectopic pregnancy incidence, from a low of 0.005 ectopic pregnancies per 1000 woman years of oral contraception or vasectomy to a high of 2.6 per 1000 woman years of no contraception. These estimated incidence rates should be useful for clinicians and patients seeking to better understand the risks and benefits of contraceptives. (Am J Obstet Gynecol 1990;163:1120-3.)

Key words: Pregnancy, ectopic; contraception; intrauterine contraceptive device; family planning

The incidence of ectopic pregnancy has increased dramatically over the last 20 years.[1-3] Possible explanations include the increased prevalence of pelvic inflammatory disease and sexually transmitted diseases, increased clinical suspicion and improved early diagnostic techniques for ectopic pregnancy (quantitative serum pregnancy hormone tests, ultrasonography, laparoscopy), the trend toward delaying childbearing until older ages when ectopic pregnancy becomes a greater risk, and the role of contraceptive practice.[1, 3-8] Studies of the association of ectopic pregnancy with contraceptive methods, in particular, the intrauterine contraceptive device (IUD),[3, 5, 6] have generated a conflicting array of results that may be a source of confusion for clinicians desiring to provide the best contraceptive alternatives to their patients.

Most studies of the possible association of contraception and ectopic pregnancy have used the case-control study design because it is the most feasible method for studying rare diseases.[9] Such a study design compares persons who have the disease in question (cases) with persons free of the disease (controls). Crucial to interpreting the results are that the controls be chosen from the same population of persons as the cases and that the controls have the potential for being exposed to the same risk factors as the case group. Selecting a control group for a case-control study of ectopic pregnancy is not a simple matter[10, 11]; some researchers have selected obstetric patients, and others nonpregnant women, as controls,[3] leading to substantially different results.[3, 5, 6, 11]

For example, in a study about the use of the IUD, the selection of the control group may determine whether the IUD appears to protect against ectopic pregnancy or to increase the risk of ectopic pregnancy; relative risk estimates range from 0.5 to 32, depending on study design.[3] To illustrate, if obstetric patients with intrauterine pregnancies are selected as controls, a low percentage of these women will have conceived while using the IUD because the IUD largely prevents intrauterine implantation. In addition, the spontaneous abortion rate is elevated among intrauterine pregnancies that result from IUD failure, and the likelihood of an induced abortion among IUD users who do not spontaneously abort also may be high. Thus women with second- or third-trimester intratuerine pregnancies are less likely than the general population to have been using an IUD. Comparing these women with those who have an ectopic pregnancy may therefore yield an elevated risk associated with IUD use among women in the case group, even if their prevalence of IUD use is not particularly high. In contrast, if nonpregnant

From the Division of Reproductive Health, Center for Chronic Disease Prevention and Health Promotion,[a] and the Division of Sexually Transmitted Diseases, Center for Prevention Services,[b] Centers for Disease Control.
Received for publication November 29, 1989; revised May 2, 1990; accepted May 18, 1990.
Reprint requests: Adele Franks, MD, Mailstop FO5, OSA/CCDPHP, Centers for Disease Control, 1600 Clifton Road, Atlanta, GA 30333.
6/1/22474

Table I. Estimated incidence rates of ectopic pregnancy by contraceptive method

Contraceptive method	*Lowest expected pregnancy rate per 1000 woman years** (A)	*Proportion ectopic implantation** (B)	*Estimated ectopic pregnancy incidence rate per 1000 woman years* (A × B)
Oral contraceptive†	1	0.005	0.005
Vasectomy	1	0.005	0.005
Condom	20	0.005	0.100
Diaphragm	30	0.005	0.150
Tubal sterilization	2	0.159	0.318
IUD‡	20	0.051	1.020
None	520	0.005	2.600

*See material and methods section for origin of these numbers.
†Combination estrogen-progestin only.
‡Unmedicated only.

women are selected as controls, the opposite conclusion is likely. Nonpregnant, sexually active women are more likely to be using contraception, and thus they may have a higher prevalence of IUD use than women in the case group, leading one to conclude that IUD use is protective against ectopic pregnancy. Interpretation of these results must be limited in scope to the comparisons actually studied. Thus no general statement may be made about whether IUD users are at increased or decreased risk of ectopic pregnancy.

For an individual clinician or patient to use the information from case-control studies in selecting a contraceptive method would be, at best, a challenging endeavor. Instead, knowing the actual rates of ectopic pregnancy among cohorts of women using various contraceptive methods might prove more useful. Unfortunately, such information can be acquired only by follow-up of large numbers of women over time (cohort study design), a process that is expensive and time-consuming. Such studies are therefore rare. Two cohort studies conducted in the 1960s and early 1970s in the United States and the United Kingdom provide sufficient information on ectopic pregnancy rates among IUD users.[12, 13] Little information exists on ectopic pregnancy rates associated with other contraceptive methods.

In this article we estimate the incidence rates of ectopic pregnancy per 1000 woman years of using various contraceptive methods to clarify the magnitude of ectopic pregnancy risk and to determine the relative ranking of risk for each contraceptive method.

Material and methods

To calculate estimated rates of ectopic pregnancy per 1000 woman years of use for each contraceptive method, we assumed that the ectopic pregnancy rate was the product of two factors: (1) the incidence rate of pregnancy (i.e., the conception rate) per 1000 woman years and (2) the proportion of pregnancies implanting in an ectopic location (outside the uterine fundus).

For women using contraception the pregnancy rate per 1000 woman years was taken as the "lowest expected" ("method") failure rate associated with the use of each contraceptive method per 1000 women in the first year of use.[14] This rate is based on the assumption of consistent and correct use of the method for the entire year. For women not using contraception, the pregnancy rate per 1000 woman years was taken as the average annual pregnancy rate per 1000 women not using contraception.[15]

For IUD users we derived the proportion of pregnancies that implant in an ectopic location from the two large cohort studies (mentioned earlier) in which, at the time of publication, more than 98% of the women enrolled used nonmedicated devices.[12, 13] The proportion of pregnancies that were ectopic among the women using IUDs (0.043 in the U.S. study and 0.072 in the U.K. study) were averaged (directly weighted by the number of woman years of IUD use in each study), yielding 0.051 for our calculations.

For women who used combination oral contraceptives, who used barrier methods, and who were partners of vasectomized men, we assumed that the contraceptive method did not affect the site of implantation; therefore we assumed that the proportion of pregnancies implanting in an ectopic location was 0.005, approximately equal to the overall proportion among reported pregnancies in the U.S. population during the early 1970s.[16] For women with tubal sterilization failures we obtained the proportion with ectopic implantation from literature reviews.[7, 8]

The IUD was the only contraceptive method for which actual observed rates of ectopic pregnancy per 1000 woman years of use were available from large cohort studies.[12, 13] These empiric rates were averaged (directly weighted by number of woman years of use in each study) and were compared with those calculated as described above.

Results

The resulting estimated ectopic pregnancy rates by contraceptive method vary more than 500-fold from the lowest rate of 0.005 per 1000 woman years among

women who used combination oral contraceptives or who were partners of vasectomized men, to the highest rate of 2.6 per 1000 woman years among women using no contraception (Table I). Thus, although using an IUD carries a higher rate of ectopic pregnancy than using barrier methods, sterilization, or oral contraception, failure to use any contraception has the highest estimated rate of ectopic pregnancy.

For IUD use, the calculated ectopic pregnancy rate can be compared with the actual rates of 1.0 per 1000 woman years in the U.S. study and 1.2 per 1000 woman years in the U.K. study referred to previously.[12, 13] On the basis of these numbers, the weighted average of 1.06 per 1000 woman years compares favorably with the calculated rate of 1.02 per 1000 woman years derived by multiplying the pregnancy rate and the proportion of resulting pregnancies that were ectopic (Table I).

Comment

We believe that expressing risk of disease, whenever possible, in terms of absolute rates of occurrence, rather than only in relative terms, is more useful for clinical decision making. For ectopic pregnancy we believe relative risk estimates from case-control studies are especially confusing because the choice of control group profoundly affects interpretation of results.

To estimate rates of occurrence of ectopic pregnancy in the absence of sufficient cohort data, we assumed that the proportion of pregnancies implanting in an ectopic location did not differ among women who used combination oral contraceptives, women who used barrier methods, women who were partners of vasectomized men, and women who used no contraception. If this assumption is incorrect, our resulting estimates of incidence rates for ectopic pregnancy might be incorrect as well; however, on the basis of current knowledge, we know of no reason to question this assumption. For those who prefer to make different assumptions regarding the likelihood of ectopic implantation, our methods may be easily applied, by substituting different numbers.

Similarly, although we used the "lowest expected" ("method") failure rates for the various contraceptive groups for our calculations, one could easily use the "typical" ("user") failure rates among women using contraception instead. While the former is based on the assumption of consistent and correct use of the contraceptive method, the latter is based on "typical" couples, including those who do not use the contraceptive method consistently or correctly. To calculate ectopic pregnancy rates based on "typical" failure rates, one could substitute pregnancy rates of 30 per 1000 women for oral contraceptives, 1.5 per 1000 women for vasectomy, 120 per 1000 women for condoms, 180 per 1000 women for the diaphragm, 4 per 1000 women for tubal sterilization, and 60 per 1000 women for the IUD.[14] This substitution results in estimated "typical" ectopic pregnancy rates of 0.15, 0.0075, 0.60, 0.90, 0.64, and 3.06 per 1000 woman years of use, respectively.

It should be noted that we used the first-year failure rates for women who used the various contraceptives while we used the average annual pregnancy rate for women who used no contraception. Because failure rates tend to decline over time for reversible contraceptives, our results may be biased in the direction of exaggerating the ectopic pregnancy risk among women using contraception. For women using sterilization to avoid pregnancy, failure rates over time may depend on method of occlusion and other factors that are not yet fully understood. Therefore predicting the direction of any potential bias in our calculations regarding sterilization is difficult.

We also assumed the ectopic pregnancy rate to be the product of the pregnancy rate and the likelihood of ectopic implantation. Again, no data exist to challenge this assumption, and our own estimate of the ectopic prengancy rate among IUD users on the basis of this multiplicative assumption compares favorably with the actual rates reported in two large cohort studies.

The only available cohort data reflect rates of ectopic pregnancy among IUD users during the 1960s and early 1970s. To make meaningful comparisons, we estimated the occurrence of ectopic pregnancy for other methods on the basis of data as close in time as possible to the IUD cohort studies. Note, however, that the currently used copper-containing IUDs have lower failure rates than the nonmedicated IUDs,[14] which were predominantly used in the 1960s and early 1970s. Thus the ectopic pregnancy rate associated with IUD use may be somewhat reduced in recent years. On the other hand, recent information suggests that the proportion of ectopic pregnancies among pregnancies resulting from tubal sterilization failure may vary according to method of occlusion and may be higher than previously believed, especially for pregnancies occurring after tubal sterilization by electrocoagulation.[17] Further research is necessary to clarify this issue.

Though our estimates are imprecise, and changes in risk over time may have occurred, the differences in magnitude of ectopic pregnancy risk between contraceptives are so large that we believe the relative ranking of risk to be correct. We hope that expressing the risk of ectopic pregnancy associated with contraceptive methods as rates of occurrence rather than as relative risks will be meaningful in the clinical setting. In particular, the fact that ectopic pregnancy risk appears to differ little between women who use IUDs and women who use no contraception should be reassuring for

those women for whom the IUD is otherwise the best contraceptive option.

REFERENCES

1. Lawson HW, Atrash HK, Saftlas AF, Franks AL, Finch EL, Hughes JM. Ectopic pregnancy surveillance, United States, 1970-1985. MMWR 1988;37(SS-5):9-18.
2. Beral V. An epidemiological study of recent trends in ectopic pregnancy. Br J Obstet Gynaecol 1975;82:775-82.
3. Chow WH, Daling JR, Cates W Jr, Greenberg RS. Epidemiology of ectopic pregnancy. Epidemiol Rev 1987;9: 70-94.
4. Robinson N, Beral V. Risk of ectopic pregnancy. Lancet 1979;2:1247-8.
5. Ory HW. Ectopic pregnancy and intrauterine contraceptive devices: new perspectives. Obstet Gynecol 1981;57: 137-44.
6. Marchbanks PA, Annegers JF, Coulam CB, Strathy JH, Leonard TK. Risk factors for ectopic pregnancy: a population-based study. JAMA 1988;259:1823-7.
7. Tatum HJ, Schmidt FH. Contraceptive and sterilization practices and extrauterine pregnancy: a realistic perspective. Fertil Steril 1977;28:407-21.
8. DeStefano F, Peterson HB, Layde PM, Rubin GL. Risk of ectopic pregnancy following tubal sterilization. Obstet Gynecol 1982;60:326-30.
9. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic research: principles and quantitative methods. New York: Van Nostrand Reinhold, 1982:62-70.
10. Weiss NS, Daling JR, Chow WH. Control definition in case-control studies of ectopic pregnancy. Am J Public Health 1985;75:67-8.
11. Weiss NS, Daling JR. Risk factors for ectopic pregnancy. JAMA 1988;260:1553-4.
12. Vessey MP, Yeates D, Flavel R. Risk of ectopic pregnancy and duration of use of an intrauterine device. Lancet 1979;2:501-2.
13. Lehfeldt H, Tietze C, Gorstein F. Ovarian pregnancy and the intrauterine device. AM J OBSTET GYNECOL 1970;108: 1005-9.
14. Trussell J, Kost K. Contraceptive failure in the United States: a critical review of the literature. Stud Fam Plann 1987;18:237-83.
15. Sheps MC. An analysis of reproductive patterns in an American isolate. Popul Stud 1965;19:65-80.
16. Centers for Disease Control. Ectopic pregnancy—United States, 1986. MMWR 1989;38:481-4.
17. Bhiwandiwala PP, Mumford SD, Feldblum PJ. A comparison of different laparoscopic sterilization occlusion techniques in 24,439 procedures. AM J OBSTET GYNECOL 1982;144:319-31.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Calcium supplementation during pregnancy may reduce preterm delivery in high-risk populations

Jose Villar, MD,[a] and John T. Repke, MD[b]
Bethesda and Baltimore, Maryland

Results are presented of a randomized, double-blinded controlled clinical trial of calcium supplementation (2.0 gm of elemental calcium as calcium carbonate) and a placebo. All participants were 17 years of age or less and clinically healthy. Patients were enrolled by the twenty third week of gestation. The mean duration of calcium supplementation or placebo was approximately 14 weeks. Treatment consisted of 2.8 (±1.5) tablets per day in the placebo group ($N = 95$) and 3.0 (±1.4) tablets per day in the calcium group ($N = 94$). Dietary calcium intake was similar in both groups at about 1200 mg/day. The calcium group had a lower incidence of preterm delivery (<37 weeks; 7.4% vs 21.1%; $p = 0.007$); spontaneous labor and preterm delivery (6.4% vs 17.9%; $p = 0.01$); and low birth weight (9.6% vs 21.1%; $p = 0.03$). This effect was also present after stratified analysis by level of treatment compliance, urinary tract infection, and chlamydial infection. Life-table analysis demonstrated an overall shift to a higher gestational age in the calcium group compared with the placebo group (log-rank test, $p = 0.02$). As suggested previously, the observed effect could be mediated by a reduction in uterine smooth muscle contractibility. If confirmed by future research, these results could represent an important preventive intervention for prematurity in high-risk populations. (AM J OBSTET GYNECOL 1990;163:1124-31.)

Key words: Calcium supplementation prematurity, high-risk population

Prematurity and low birth weight are among the leading causes of perinatal and infant mortality in both developing and developed populations.[1, 2] Therefore their prevention, even if achieved at modest proportions, would have major health implications.

We previously reported blood pressure reduction among pregnant and nonpregnant women who received calcium supplementation, observations that are supported by similar evidence from hypertensive subjects.[6] Two recently published literature reviews summarized these findings.[6, 7]

It has been postulated that the blood pressure–lowering effect of calcium supplementation involves mechanisms related to parathyroid hormone levels, negative calcium balance, and plasma renin activity.[6, 8] These proposed mechanisms would ultimately influence intracellular free (cytosolic) calcium concentrations in smooth muscle cells, [6] thus reducing smooth muscle cell tone and increasing its relaxation. Therefore high calcium intake would reduce blood pressure by reducing smooth muscle reactivity. If this is the case, a similar effect should be present at the level of the uterine muscle, particularly in the third trimester of pregnancy when the uterus represents the largest mass of smooth muscle in mammals. Interestingly, there are data to support an association between pregnancy-induced hypertension, preeclampsia, and increased uterine contractibility.[9, 10]

We previously hypothesized that calcium supplementation during pregnancy can reduce smooth muscle contractibility and tone[6] and that this effect can be clinically manifested by a reduction in blood pressure and a reduction in the incidence of premature labor and delivery.

This article presents the results of a randomized, double-blinded placebo-controlled clinical trial of the effect of calcium supplementation during pregnancy (2.0 gm/day) on the incidence of prematurity and preterm labor. The a priori hypothesis that calcium supplementation reduces the incidence of prematurity (<37 weeks) is tested.

Material and methods

This randomized clinical trial was conducted at the Adolescent Pregnancy Clinic of The Johns Hopkins Hospital in Baltimore between 1985 and 1988. All pa-

From the Prevention Research Program, National Institute of Child Health and Human Development, National Institutes of Health,[a] and the Department of Gynecology and Obstetrics, The Johns Hopkins Hospital.[b]
Supported by grants to Dr. Repke from the National Dairy Board and the National Dairy Council.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Received for publication January 3, 1990; accepted May 18, 1990.
Reprint requests: John T. Repke, MD, Department of Gynecology and Obstetrics, The Johns Hopkins Hospital, 600 N. Wolfe St., Houck 228, Baltimore, MD 21205.
6/1/22483

tients were 17 years of age or younger at the time of recruitment. Most of the subjects were nulliparous [84 in the calcium group, 78 placebo group (NS)] with a known last menstrual period and singleton pregnancies. All were free of any underlying medical disorders, determined by history, physical examination, and laboratory tests.

Patients were contacted during the first prenatal visit about participation in the project. By the twentieth week of gestation enrollment to the study was offered and parental consent or patient informed consent was obtained. If patients agreed to participate they were randomized to the calcium and placebo group at the next prenatal visit. A computer-generated list of random numbers was used to allocate the corresponding treatments. Opaque envelopes with the bottles numbered were located at the clinic and the project coordinator was in charge of the administration of the treatment assigned. Prenatal care was carried out according to the protocols of The Johns Hopkins Adolescent Pregnancy Program, and under the direct supervision of its medical director (J. T. R). The study protocol was approved by The John Hopkins Hospital's Joint Committee on Clinical Investigation. Patients received a small monetary compensation for travel and compliance with the study protocol.

The calcium supplementation group received four tablets of calcium carbonate (OSCAL 500, Marion Laboratories, Kansas City, Mo.) per day. Each tablet provides 500 mg of elemental calcium for a total of 2.0 gm/day. This dosage was based on our previous evidence on the effect of calcium supplementation on blood pressure during pregnancy.[4,8] The placebo group received four tablets of the same weight, size, color, and organoleptic characteristics as the calcium tablets. All of the tablet containers were prepared at The Johns Hopkins Hospital Pharmacy. Patients were prescribed prenatal vitamins containing 200 mg of calcium and 100 mg of magnesium per day.

Total intake, including a detailed description of calcium-containing food stuffs, was evaluated three times during pregnancy by a senior nutritionist by means of food patterns and 24-hour recall methods. These data were further analyzed with regard to specific nutrient content.

Treatment compliance was assessed at each visit by the project coordinator. Patients were questioned during each visit about the number of tablets taken and the project coordinator counted the number of tablets left in the container as many times as possible during pregnancy. The number of tablets taken of the total number of tablets required to be taken between prenatal visits (compliance rate) was calculated for the calcium and placebo groups. With that information and the time elapsed between visits, the actual number of tablets reported taken per day was calculated for three periods during pregnancy for the two treatment groups.

Gestational age was calculated using the best obstetric estimation by the attending obstetrician who was totally unaware of the treatment groups. This evaluation was done at the time of randomization and was corroborated during pregnancy. It includes last menstrual period information (consideration menstrual patterns and contraceptive use), several standard ultrasonographic measurements between 16 and 20 weeks, or at the time of the first visit, fundal height. If the gestational age based on the last menstrual period and the ultrasonographic estimation differed more than 2 weeks, the ultrasonogram was used for gestational age calculation; otherwise gestational age was based on the last menstrual period.

The incidence of the following events was calculated for the analysis: *preterm labor without delivery*, diagnosed by obstetricians unaware of the treatment group and based on uterine activity of at least four contractions in 20 minutes or eight contractions in 1 hour after the patient was admitted to the hospital and discharged after 24 hours without delivery; *preterm labor and delivery* during the same hospital admission; *preterm delivery* (<37 weeks); *delivery between 30 and 37 weeks; idiopathic prematurity*, including only preterm (<37 weeks) deliveries without previous medical or obstetric complications; *spontaneous prematurity*, including only preterm deliveries with spontaneous onset of labor regardless of vaginal or cesarean section delivery.

The hypothesis tested was whether provision of calcium supplementation (2 gm/day) to high-risk pregnant adolescents reduces uterine muscle tone and contractions, resulting in a lower rate of prematurity. A sample size of 95 subjects in each group has a statistical power of 80% to detect a reduction in prematurity (<37 weeks) rates from 20% to 7% at an α-level of 0.05. The expected 20% incidence of prematurity in the placebo group is obtained from values previously reported from The Johns Hopkins adolescent population.[11] All subjects were included in the treatment group to which they were originally randomized regardless of any follow-up and treatment compliance experiences.

The incidence of preterm delivery and all other secondary outcomes were compared between treatment groups using Mantel-Haenszel χ^2, Fisher's exact test, or t test, when required. To evaluate the treatment effect on the complete gestational age distribution, a life-table analysis was performed with calcium and placebo treatments as the independent variable and the delivery at different gestational ages as the outcome variable. The life-table analysis was performed for all patients, including dropouts up until the time they left the study. Life tables were compared with the log-rank test. Two-tailed p values are presented.

Table I. Population characteristics by treatment group

	Calcium (N = 95)	*Placebo (N = 95)*	*p Value*
Maternal age (yr) (mean ± SD)	16.2 ± 0.8	16.3 ± 0.9	NS
Education (yr) (mean ± SD)	9.3 ± 1.3	9.4 ± 1.5	NS
No. of prenatal visits (mean ± SD)	10.2 ± 3.7	10.0 ± 4.2	NS
Maternal height (in) (mean ± SD)	64.6 ± 10.9	65.8 ± 14.9	NS
Black race	92.6	94.7	NS
Marital status single (%)	97.9	98.9	NS
Previous preterm delivery (<37 wk) (%)	2.1	2.1	NS
Previous low birth weight (≤2500 gm) (%)	2.1	2.1	NS
Previous pregnancy-induced hypertension (%)	2.1	2.1	NS
Spontaneous abortions (%)	5.3	8.4	
Induced abortions (%)	15.2	18.9	NS
Primigravid (%)	70.5	61.0	NS
Nulliparous (%)	88.4	82.1	NS

Calcium supplementation of 2.0 gm/day of elemental calcium.

Table II. Population characteristics at the time of randomization

	Calcium (N = 95)	*Placebo (N = 95)*	
Gestational age (wk)	23.7 ± 4.3	23.4 ± 3.8	NS
Uterine height (cm)	23.7 ± 4.1	23.6 ± 3.8	NS
Maternal weight (kg)	63.0 ± 10.6	67.4 ± 13.5	$p < 0.01$
Systolic blood pressure (mm Hg)	103.1 ± 9.2	102.4 ± 10.4	NS
Diastolic blood pressure (mm Hg)	60.9 ± 8.8	61.3 ± 8.0	NS

Values are mean ± SD. Calcium supplementation of 2.0 gm/day of elemental calcium.

Results

Table I presents the baseline population characteristics for the 190 study patients. No statistically significant differences were observed for all variables. Table II presents patient characteristics at the time of randomization. No differences between groups were detected with the exception of maternal weight. The calcium group had lower mean maternal weight (63.0 ± 10.6 kg) than the placebo group (67.4 ± 13.5 kg; $p < 0.01$).

There was a tendency to a higher incidence of bacteriuria (11.7% vs 5.4%; NS) and symptomatic chlamydia vaginitis (7.4% vs 3.2%, NS) in the placebo group compared with the calcium group. No differences were observed in the incidence of either pyelonephritis (1.1% for calcium group vs 2.1% for placebo group, NS), or sexually transmitted diseases (14.7% for calcium vs 15.8% for placebo group NS).

Table III shows data on the treatment compliance for the two study groups. Both groups started treatment at similar gestational age and continued under treatment for similar length of time (14.1 ± 4.4 weeks for calcium group vs. 13.2 ± 4.9 weeks for placebo group, NS). The actual number of tablets reported taken was also similar in both groups at different points in gestation and for the total follow-up period; overall, patients took a daily average of three tablets per day (75% of the expected number of tablets).

Selected nutrient intakes at baseline, during the third trimester of pregnancy, and for the overall study period (average of all intakes recorded per patient) by treatment group are presented in Table IV. No differences were observed between groups for the nutrients evaluated. Mean calcium intake for both groups was approximately 1200 mg/day throughout pregnancy.

The calcium supplementation group had a lower incidence of preterm labor and delivery (7.4% vs 16.8%; $p < 0.05$), preterm labor without delivery (3.2% vs 7.4%; NS), preterm delivery <37 weeks, (7.4% vs 21.1%; $p = 0.007$), preterm delivery ≥30 but <37 weeks (4.4% vs 15.7%; $p < 0.01$), low birth weight (≤2500 gm, 9.6% vs 21.1%; $p = 0.03$), and preterm

Table III. Treatment compliance by supplementation groups

	Calcium (N = 91)	*Placebo (N = 92)*	*p Value*
Period under treatment 1 week	14.1 ± 4.4	13.2 ± 4.9	NS
Actual no. of tablets taken per day			
Total period	3.0 ± 1.4	2.8 ± 1.5	NS
<30 wk	3.1 ± 1.4	2.8 ± 1.3	NS
30-34 wk	2.7 ± 1.5	2.9 ± 1.4	NS
>34 wk	3.1 ± 1.5	2.8 ± 1.6	NS

Values are mean ± SD. Ideal number of tablets per day = 4 (500 mg of elemental calcium each tablet). Three patients in the placebo group and four in the calcium group abandoned treatment at different times during the study and complete compliance information could not be obtained.

Table IV. Estimated daily nutrient intakes by treatment group

	Baseline	*Third trimester*	*Overall**
Protein			
Calcium group	84 ± 34	90 ± 29	89 ± 27
Placebo group	91 ± 38	90 ± 43	92 ± 35
Calories (kcal)			
Calcium group	2228 ± 873	2436 ± 849	2363 ± 680
Placebo group	2285 ± 866	2300 ± 1066	2314 ± 782
Iron (mg)			
Calcium group	11 ± 5	15 ± 7.6	13 ± 4.3
Placebo group	13 ± 6	13 ± 7.3	13 ± 5.6
Vitamin D			
Calcium group	5.7 ± 3.9	6.5 ± 4.2	6.9 ± 5.4
Placebo group	7.4 ± 5.7	7.5 ± 10	7.3 ± 4.9
Calcium (mg)			
Calcium group†	1119 ± 677	1220 ± 536	1201 ± 521
Placebo group	1336 ± 796	1122 ± 655	1248 ± 610
Vitamin C (mg)			
Calcium group	224 ± 192	226 ± 161	224 ± 131
Placebo group	253 ± 164	241 ± 170	230 ± 120

Intakes determined by 24-hour dietary recall.
*Mean intake per patient during study period.
†Supplemental calcium not included.

delivery after spontaneous labor (6.4% vs 17.9%; $p = 0.01$). No effect was observed on the incidence of intrauterine growth retardation and premature rupture of membranes (<37 weeks, >24 hours before initiation of labor; Table V).

All seven preterm deliveries in the calcium group were associated with medical complications ($n = 3$), vaginal infections ($n = 6$), sexually transmitted diseases ($n = 2$), premature rupture of membranes ($n = 2$), proteinuria ($n = 1$), and pregnancy-induced hypertension ($n = 1$). Fourteen of the preterm deliveries in the placebo group associated were also associated with these complications. Therefore the incidence of idiopathic prematurity is 0 of 94 patients in the calcium group and 6.3% in the placebo group ($p = 0.01$; Table V).

The mean duration of labor was longer in the calcium group (12.0 ± 7.4 hours than in the placebo group (9.9 ± 6.2 hours; $p = 0.05$). Gestational age at birth was 1.3 weeks longer ($p = 0.01$) and infants were 189 gm ($p = 0.06$) heavier in the calcium group than in the placebo group (Table VI).

The incidence of pregnancy-induced hypertension was 1.7 times higher in the placebo group than in the calcium group (NS), and the incidence of preeclampsia was 3.4% in the placebo group with no cases observed in the calcium group ($p = 0.10$; Table VI).

When only those patients with vaginal deliveries are included in the analysis the incidence of preterm delivery (<37 weeks) was 6.5% for the calcium group ($n = 77$) and 16.2% for the placebo group ($n = 74$; $p = 0.05$); the values for low birth weight (≤2500 gm) in this subgroup were 11.2% and 20.5% ($p = 0.08$) for the calcium and placebo groups, respectively.

Although the differences in the proportion of bacteriuria and chlamydia vaginitis between groups were not statistically significant, it was thought that the observed tendency could influence the results. A stratified

Table V. Pregnancy outcome by treatment group

	Calcium (N = 94) (%)	*Placebo (N = 95) (%)*	*p Value*
Preterm labor and delivery	7.4	16.8	0.05
Preterm labor without delivery	3.2	7.4	NS
Preterm delivery (<37 wk)*	7.4	21.1	0.007
Preterm delivery (>30 but <37 wk)	4.4†	15.7‡	0.01
Idiopathic prematurity§	0.0	6.3	0.01
Spontaneous prematurity‖	6.4	17.9	0.01
Low birth weight (≤2500 gm)	9.6	21.1	0.03
Postdates (>42 wk)	7.4	5.3	NS
Intrauterine growth retardation	3.2	3.2	NS
Premature rupture of membranes	2.1	1.0	NS

Premature rupture of membranes: <37 weeks; >24 hours before the initiation of labor.

*Gestational age as best obstetric estimation including last menstrual period, ultrasonography between 16 and 20 weeks or at first prenatal visit, and fundal height.

†$N = 91$.

‡$N = 89$.

§Idiopathic prematurity: Only preterm deliveries without any previous medical or obstetric complications.

‖Spontaneous prematurity: Preterm deliveries whose labor onset was spontaneous regardless of route of delivery.

Table VI. Pregnancy outcome by treatment group

	Calcium (N = 94)	*Placebo (N = 95)*	*p Value*
Apgar score (≤7 at 5 min) (%)	4.4	10.5	0.004
Cesarean section (%)	17.0	17.9	NS
Pregnancy-induced hypertension* (%)	3.3	5.7	NS
Preeclampsia* (%)	0.0	3.4	0.10
Duration of labor (hr) (mean ± SD)	12.0 ± 7.4†	9.9 ± 6.2‡	0.05
Gestational age (wk) (mean ± SD)	39.2 ± 2.7	37.9 ± 3.9	0.01
Weight (gm) (mean ± SD)	3128.6 ± 598	2939.4 ± 784	0.06
Length§ (cm) (mean ± SD)	50.1 ± 3.4	49.4 ± 4.8	NS

Pregnancy-induced hypertension: Diastolic blood pressure at ≥30 weeks >90 or ≥140/90 mm Hg. Preeclampsia: ≥300 mg proteinuria at >30 weeks and pregnancy-induced hypertension.

*Calcium: $N = 90$. Placebo: $N = 88$.

†$N = 90$.

‡$N = 80$.

§$N = 87$.

analysis was therefore performed by infection status. Among the noninfected patients, the incidence of prematurity was 8.1% in the calcium group ($n = 86$) and 24.7% in the placebo group ($n = 77$; $p = 0.003$). The small number of patients in the infected groups precludes any further consideration of the calcium effect among them.

The effect of calcium supplementation on the incidence of prematurity was further studied by level of treatment compliance. Three patients in the placebo group and four in the calcium group did not have complete compliance information and are not included in this analysis. In the high-compliance calcium supplementation group (>50% of ideal treatment; $n = 49$), no cases of prematurity were observed, whereas the preterm rate was 9.5% in the high-compliance placebo group ($n = 42$; $p = 0.04$). The incidence of prematurity was also lower in the low-compliance calcium group (<50% of ideal treatment; $n = 41$; 17.1%) compared with the low-compliance placebo group ($n = 50$; 26.0%; NS). These data suggest an independent effect on prematurity of both calcium supplementation and treatment compliance.

Fig. 1 presents the cumulative probability of delivery (8 percent) for two treatment groups at different periods of gestation. All participants (including one drop-out until the time she left the study) are included. Information is derived from life-table analysis using the Kaplan-Meier method Statistical Analysis Systems test procedure; differences between the two life tables have

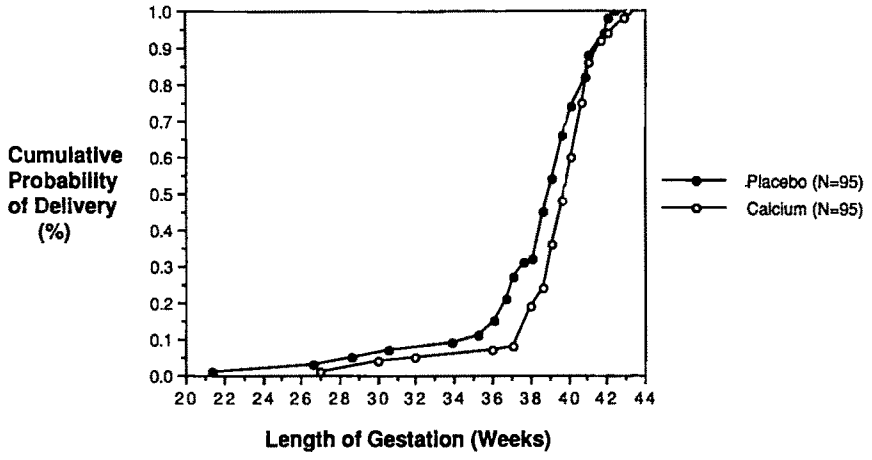


Fig. 1. Calcium supplementation and prematurity. Cumulative probability of delivery (percent) from life-table analysis for each treatment group. At all gestational ages the calcium group has lower risk of delivery than the placebo group (log-rank test, $p = 0.02$)

been statistically tested with the log-rank and Wilcoxon tests. The calcium group had lower risk of delivery at all gestational ages than the placebo group ($p = 0.02$) (Fig. 1). Finally, urinary and chlamydial infections as well as prepregnancy weight, the identified possible confounding variables, were not associated with prematurity when included in the life-table analysis ($p = 0.10$).

Comment

We have presented evidence from a double-blind, randomized placebo-controlled clinical trial of the effect of calcium supplementation on the incidence of preterm delivery. Groups were similar in several baseline characteristics known to be related to preterm delivery.

The calcium group had lower maternal weight at the time of randomization; however, if an effect was present, it would be in the direction of increasing prematurity or low birth weight in this group. Exclusion of patients with urinary or vaginal infections or inclusion of these variables in the life-table analysis demonstrated that the observed differences in prematurity rates between treatment groups could not be explained by these baseline differences.

Nutrient intakes were similar in both groups and the mean calcium intake was approximately 75% of the recommended level for pregnant adolescents at the time the study was conducted.[12]

As expected among these high-risk adolescents, treatment compliance showed large variability. However, the calcium effect was observed at both compliance levels with the strongest effect among those patients with higher compliance rates. Interestingly, treatment compliance, a proxy indicator for adherence to medical care in general, education, or personal motivation also had an independent effect preventing preterm delivery.

Finally, patients in the calcium group, in agreement with previous reports,[4, 13] had a lower incidence of preeclampsia and pregnancy-induced hypertension without reaching statistically significant levels because of small sample size. Although it is possible that part of the prematurity effect could be mediated through this mechanism, it is unlikely to explain an effect of this magnitude. We expect to evaluaute this association in more detail in a larger, multicenter clinical trial.

Therefore, we believe that the observed results on preterm delivery are unbiased and are likely to represent a true biologic effect. More data are needed to confirm this evidence. If the results are valid, obstetricians could incorporate this relatively easy and inexpensive preventive action into the prenatal care of high-risk poplations.

A recent report[13] provided additional information on the effect of 2 gm of elemental calcium supplementation during pregnancy. This study confirmed the previously reported blood pressure effect.[4] The percentage of deliveries between 36 and 38 weeks was 10.9% for the calcium group and 15.7% for the placebo group. Similar to our study, the calcium group had a higher mean birth weight (265 gm difference) and a statistically significant longer gestational age (39.3 weeks in the calcium group and 38.7 weeks in the placebo group; ($p < 0.05$).[13] Therefore, these data would seem to support the results presented in this article.

Currently, we do not have definitive physiologic explanations of the mechanisms involved in these observed effects. The role of ionized calcium in smooth muscle contraction, in uterine muscles,[14, 15] and vascular tissues[16, 17] has been extensively studied, including alterations in calcium concentration associated with hy-

pertension.[18] We[3] and others[4, 7] have demonstrated blood pressure reduction with calcium supplementation, and this effect is suggested to be mediated by induced changes at the vascular smooth muscle level.[6]

Therefore, it is logical to expect that a similar action will be observed at the uterine level, particularly near term when the uterus represents the largest mass of smooth muscle in mammals. We do not think that high calcium intake is responsible for affecting uterine contractibility by a direct effect on uterine extracellular ionized calcium. It is more likely that high calcium intake is related to an effect on membrane calcium channels, particularly those controlled by hormones.[15]

For instance, high calcium intake, as we recently have proposed,[6, 8] could participate in these mechanisms by reducing the level of parathyroid hormone, thus lowering intracellular free calcium levels and increasing smooth muscle relaxation. The parathyroid hormone effect on intracellular concentration of calcium has been demonstrated in several types of cells.[6, 19-2] Two mechanisms can mediate this action: an increase in cellular membrane permeability to calcium and an activation of adenyl cyclase and an increase of cyclic adenosine monophosphate with a consequent liberation of calcium from the mitochondria to the cytosol.[6]

Seemingly paradoxically, calcium channel blockers are also known to reduce blood pressure and uterine contractibility[22] and have been suggested for the treatment of preterm labor[23] and preterm labor in hypertensive patients.[24] It has been recently shown that diltiazem, a calcium channel blocker, lowers serum parathyroid hormone in vivo and in vitro in a dose-dependent fashion.[25] This observation suggests that lowering the level of pregnancy-induced hypertension could be the mechanism explaining the similar effects of high calcium intake and calcium channel blockade on smooth muscle contractibility.

Finally, recent evidence from our unit also suggests that the effect may be mediated through or by changes in the renin-angiotensin system and its interaction with pregnancy-induced hypertension.[8] In vitro studies appear to support these relationships.[26]

Clearly, the mechanisms involved in the calcium supplementation effect on smooth muscle contraction and on the incidence of prematurity require extensive physiologic research. We think, however, that on the basis of data presented in this article that the feasibility of implementing this relatively simple intervention in high-risk populations and the magnitude of the expected impact on prematurity are strong arguments in favor of further extensive clinical evaluation of this promising preventive prenatal care activity.

Linda Markush, MPH, was the project coordinator during the study period. Dr. J. M. Belizan was the consultant for the project.

REFERENCES

1. Villar J, Belizan JM. The relative contribution of prematurity and fetal growth retardation to the incidence of low birth weight in developed and developing societies. Am J Obstet Gynecol 1982;143:793-8.
2. McCormick M. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82-90, 1985.
3. Belizan JM, Villar J, Pineda O, et al. Blood pressure reduction in young adults with calcium supplementation: a randomized trial. JAMA 1983;249:1161.
4. Villar J, Repke J, Belizan JM, Pareja G. Calcium supplementation reduces blood pressure during pregnancy: results of a randomized controlled clinical trail. Obstet Gynecol 1987;70:317-22.
5. McCarron D, Morris C. Blood pressure response to oral calcium in persons with mild to moderate hypertension. Ann Intern Med 1985;103:825.
6. Belizan JM, Villar J, Repke JT. The relationship between calcium intake and pregnancy-induced hypertension: up-to-date evidence. Am J Obstet Gynecol 1988;158:898-902.
7. Parrot-Garcia M, McCarron DA. Calcium and hypertension. Nutr Rev 1984;42:205.
8. Repke JT, Villar J. Anderson C, Pareja G, Dubin N, Belizan JM. Biochemical changes associated with calcium supplementation induced blood pressure reduction during pregnancy. Am J Obstet Gynecol 160:684-90, 1989.
9. Zuspan F, Talledo E. Factors affecting delivery in eclampsia: the condition of the cervix and uterine activity. Am J Obstet Gynecol 1968;100:672-85.
10. Cobo E. Uterine hypercontractility in toxemia of pregnancy. I. Its prolonged therapeutic control. Am J Obstet Gynecol 1964;90:505-10.
11. Hardy J, King T, Repke J. The Johns Hopkins Adolescent Pregnancy Program: an evaluation. Obstet Gynecol 1987; 69:300-6.
12. National Research Council Food and Nutrition Board. Recommended dietary allowances. 9th ed. Washington DC: National Academy of Sciences, 1980.
13. Lopez-Jaramillo P, Narvaez M, Weigel RM, Yepez R. Calcium supplementation reduces the risk of pregnancy-induced hypertension in an Andes population. Br J Obstet Gynacol 1989;96:648-55.
14. Marston S. Calcium ion-dependent regulation of uterine smooth muscle thin filaments by caldesmon. Am J Obstet Gynecol 1989;160:252-7.
15. Corsten M, Miller J. A new look at uterine muscle contraction. Am J Obstet Gynecol 1987;157:1303-15.
16. Rasmussen H. The calcium messenger system. N Engl J Med 1986;314:1094-1100.
17. Rasmussen H. The calcium messenger system. N Engl J Med 1986;314:1164-70.
18. Zidek W. Heckmann U, Lasses H, Vetter H. Intracellular free Ca^{++} and Na^{+} in primary hypertension in arterial smooth muscle and in red blood cells. Am J Nephrol 1986;6(suppl 1):51-3.
19. Borle AB, Vchikawa T. Effects of parathyroid hormone on the distribution and transport of calcium in cultured kidney cells. Endocrinology 1978;90:1725-32.
20. Chausmer AB, Sherman BS, Wallach S. The effect of parathyroid hormone on hepatic cell transport of calcium. Endocrinology 1972;90:633-72.
21. Borle AB. Calcium metabolism in He La cells and the

effect of parathyroid hormone. J Cell Bio 1968;36:567-82.
22. Bird LM, Anderson NC, Chandler ML, Young R. The effect of aminophylline and nifedipine on contractility of isolated pregnant human myometrium. AM J OBSTET GYNECOL 1987;157:171-7.
23. Read MD, Wellby DE. The use of a calcium antagonist (nifedipine) to suppress preterm labour. Br J Obstet Gynaecol 1986;93:933-7.
24. Ulmsten U. Treatment of normotensive and hypertensive patients with preterm labor using oral nifedipine, a calcium antagonist. Arch Gynecol 1984;236:69-72.
25. Seely E, LeBoff M, Brown E, et al. The calcium channel blocker diltiazem lowers serum parathyroid hormone levels "in vivo" and "in vitro." J Clin Endocrinol Metab 1989;68:1007-12.
26. Schleiffer R, Gairard A. Angiotensin II enhances calcium exchanges in isolated rat aorta. Eur J Pharmacol 1985; 111:129-32.

The association of intrapartum asphyxia in the mature fetus with newborn behavior

James A. Low, MD, Darwin W. Muir, PhD, Elizabeth A. Pater, RN, and E. Jane Karchmar, BA
Kingston, Ontario, Canada

A matched cohort study of mature newborns with biochemically determined intrapartum fetal asphyxia and mature newborns with normal blood gas and acid-base assessments at delivery were studied to demonstrate the effect of fetal asphyxia on newborn behavior as expressed by the Brazelton newborn behavioral assessment scale. The newborn behavioral assessment scale was administered 3 days after delivery and again 2 weeks after delivery. The Lester newborn behavioral assessment scale summary scores for the group with asphyxia were of the same order as those in the control group. This was also true of the group of newborns with asphyxia with the more severe metabolic acidosis and those with low Apgar scores. These findings support the contention that many newborns who have undergone an intrapartum asphyxial insult will not have evidence of central nervous system injury and that the threshold of central nervous system injury is at the severe end of the spectrum of asphyxia as expressed by a metabolic acidosis. (AM J OBSTET GYNECOL 1990;163:1131-5.)

Key words: Fetal asphyxia, newborn behavior

Laboratory and clinical evidence suggest that fetal asphyxia can cause central nervous system injury and in surviving newborn infants result in motor and cognitive deficits.[1] Freeman[2] summarized the National Institutes of Health consensus conference on prenatal and perinatal factors associated with brain disorders by stating: "There appear to be degrees and durations of hypoxia tolerated without obvious effects on mental and motor function, yet, when the duration and severity of hypoxia exceed these thresholds motor dysfunction results and may be accompanied by mental retardation."

Although there are thresholds of asphyxia beyond which damage becomes manifest, these thresholds remain to be defined. A proper examination of this relationship requires an accurate diagnosis of fetal asphyxia and a reliable measure of central nervous system injury.

The diagnosis of fetal asphyxia requires a blood gas and acid-base assessment to determine a significant metabolic acidosis. The clinical criteria used in many studies are indicative but not diagnostic of fetal asphyxia because of their relatively low sensitivity and high false-positive rates.[3]

The concept of a continuum of casualty[4] implies that beyond the threshold a moderate amount of asphyxia may cause a small amount of damage, whereas severe

From the Department of Obstetrics and Gynecology, Queen's University.
Supported by Medical Research Council Grant No. MT-9834.
Received for publication December 28, 1989; revised June 6, 1990; accepted June 18, 1990.
Reprint requests: J. A. Low, MD, Department of Obstetrics and Gynaecology, Queen's University, Kingston, Ontario, Canada K7L 3N6.
6/1/23214

asphyxia may cause a large amount of damage. Clinical measures of such damage can be obtained in follow-up studies with the identification of major and minor motor or cognitive handicaps or developmental delays that emerge during infancy or early childhood.

However objective measures of central nervous system injury during the newborn period are not available. In addition, less severe asphyxial insults may be significant but behavioral index may not be sufficiently sensitive to demonstrate the effect on the central nervous system.

The Brazelton[5] neonatal behavioural assessment scale was conceived as a clinical instrument for evaluation and measurement of neonatal behavior and has been reputed to provide a sensitive index of central nervous system function. Tronick[6] proposed that behavioral assessments in the newborn period may identify mild dysfunctions that are not apparent from standard neurologic examination. For example, the degree to which the infant is able to achieve, maintain, and modulate state control during both sleep and alert periods after sensory stimulation is thought to reflect the maturity and integrity of central nervous system control of the highest order.[7] Thus the newborn behavioral assessment scale, when administered by trained examiners, may provide an objective measure of minimal central nervous system injury during the neonatal period. The effect of a number of environmental stress factors on the newborn have been demonstrated with the newborn behavioral assessment scale; examples include alcohol,[8] opiates,[9] analgesics, and anesthetic agents.[10, 11]

The objective of the present study was to examine the association of biochemically determined intrapartum asphyxia in the mature fetus with newborn behavior as expressed by the newborn behavioral assessment scale 3 days and again 2 weeks after delivery.

Methods

A matched case-control study was carried out between January 1987 and June 1989. During this period, a blood gas and acid-base assessment of umbilical cord blood at delivery was performed for 3674 patients representing approximately 70% of all deliveries. The criteria of significant intrapartum fetal asphyxia was an umbilical artery buffer base value <34.0 mmol/L.[3] Fetal asphyxia was observed in 99 newborns at delivery, an incidence of 2.7%.

The group with asphyxia in this study included 51 of the 99 newborns with a significant metabolic acidosis at delivery. Newborns not included in the study were preterm newborns and newborns whose parents either did not consent to participate or who could not be seen immediately after delivery to obtain consent. There was no significant difference between the umbilical artery buffer base value of newborns in the group with asphyxia (mean umbilical artery buffer base value 30.5 ± 4.2 mmol/L) and the asphyxiated newborns not entered in the study (mean umbilical artery buffer base value 30.4 ± 3.9 mmol/L).

The control group consisted of the next newborns with an umbilical artery buffer base value >38 mmol/L, matched for gestational age and birth weight ± 200 gm whose parents consented to participation of their newborns in the study. The mean birth weight for the group with asphyxia was 3267 gm and for the control group was 3315 gm. The mean umbilical artery buffer base value for the control group was 42.2 mmol/L.

The Apgar scores at 1 and 5 minutes were recorded for each newborn. Newborn encephalopathy was documented on the basis of observations of the nurses and physicians. The criteria for the diagnosis included behavioral changes, abnormalities of tone, and seizures. Abnormalities of behavior, irritability, jitteriness, lethargy, and coma were identified by observations of spontaneous movement of the infant. Increased or decreased tone was identified during observations of posture and active and passive movement. Subtle, tonic, or clonic seizures were noted. Newborn encephalopathy was classified as mild with abnormalities of behavior, moderate in the presence of lethargy and hypotonia, and severe in the presence of seizures.

The newborn behavioral assessment scale was administered in the hospital to newborns when they were 3 days old if medically stable and again in the home 2 weeks after delivery by a research assistant who had been certified as a reliable examiner by Brazelton. The research assistant was not informed of whether the newborn infant was of the group with asphyxia or the control group. The newborn behavioral assessment scale includes 26 behavioral items (the pin-prick item was omitted) and 16 reflexes. The Lester cluster summary scores[10] were used to summarize the newborns' performance. He combined the 26 behavioral items into six summary clusters and used the 16 reflexes to generate a seventh cluster. The six behavioral clusters are: (1) habituation, response decrement to repeated auditory, visual, and tactile stimulation; (2) orientation, orienting response toward animate and inanimate stimuli, and overall alertness; (3) motor, integrated motor acts and overall muscle tone; (4) range of state, the rapidity of build-up, peak, and lability of state changes; (5) regulation of state, infants' ability to modulate their own state control; and (6) autonomic regulation, signs of physiologic stress such as tremors, startles, and changes of skin color. The seventh or reflex cluster is the total number of intact reflexes scored.

In administering the test it was difficult to obtain complete data for all the items for all subjects because

Table I. Brazelton newborn behavioral assessment scale

	3 days					*2 weeks*				
	Asphyxia		*Normal*			*Asphyxia*		*Normal*		
Summary scores	*No.*	*Mean*	*No.*	*Mean*	*p Value*	*No.*	*Mean*	*No.*	*Mean*	*p Value*
Habituation	25	7.7	21	7.9	NS	9	7.6	9	7.6	NS
Orientation	49	7.1	49	7.0	NS	47	7.3	49	7.0	NS
Motor	51	5.1	50	5.2	NS	47	5.2	49	5.4	0.09
Range of state	51	3.8	50	3.8	NS	47	3.0	49	3.4	0.05
Regulation of state	50	5.4	50	5.4	NS	47	4.6	49	4.8	NS
Autonomic regulation	51	6.4	50	6.5	NS	47	6.6	48	6.8	NS
Reflexes	51	14.1	50	13.7	NS	47	13.1	49	13.2	NS

The Lester summary scores for the newborn behavioral assessment scale administered 3 days and 2 weeks after delivery for the group with asphyxia and the control group.

of the state control requirements. For example, habituation must be administered while the infant is asleep, and many waken after a few trials.

Statistical analysis included the Student t test. Statistical significance was $p < 0.05$.

This study was approved by an ethics review committee of the Faculty of Medicine, Queen's University.

Results

The Lester summary scores of the newborn behavioral assessment scale for the group with asphyxia and the control group administered 3 days after delivery are presented in Table I. There were no significant differences between the summary scores of the group with asphyxia and the control group. To provide a complete description of the results, average performance on the individual items, which constitute the summary scores, were compared. This more detailed analysis failed to reveal any significant differences between the two groups on individual item scores.

The Lester summary scores of the newborn behavioral assessment scale for the group with asphyxia and the control group administered 2 weeks after delivery are also presented in Table I. The summary score for range of state in the group with asphyxia was significantly lower than that in the control group and a similar trend was apparent for the motor score. A review of the individual items that constitute the summary scores identified six items for which the score of the group with asphyxia was lower than that of the control group ($p < 0.1$). In regard to the range of state, the average for all items in the group with asphyxia were lower than those in the control group, whereas in regard to motor summary, the differentiating items were pull to sit and motor maturity.

The group with asphyxia was divided into two subgroups on the basis of the severity of the metabolic acidosis. The mean umbilical artery buffer base value was 33.3 mmol/L for the group with moderate asphyxia and 27.9 mmol/L for the group with more severe asphyxia. There was no evidence that the group with more severe asphyxia had significantly lower summary scores than those of the group with moderate asphyxia.

The mean Apgar scores in the group with asphyxia were significantly lower than those in the control group at 1 minute (6.2 vs 8.2, $p < 0.0001$) and at 5 minutes (8.2 vs 9.1, $p < 0.004$). Thus an analysis of the Lester summary scores for the newborn behavioral assessment scale administered 3 days and 2 weeks after delivery in relation to Apgar scores in the group with asphyxia was carried out. Newborns with Apgar scores 0 to 3 were compared with newborns with Apgar scores 4 to 7 and Apgar scores 8 to 10 at 1 minute and 5 minutes after delivery. There were 11 newborns in the group with asphyxia with Apgar scores 0 to 3 at 1 minute after delivery. Newborns with Apgar scores 0 to 3 after delivery demonstrated a lower summary score for regulation of state ($p < 0.03$) 3 days after delivery, and a lower summary score for autonomic regulation ($p < 0.03$) 2 weeks after delivery. These differences likely reflect chance in view of the number of analyses. There were only two newborns with Apgar scores of 0 to 3 at 5 minutes after delivery. There were no significant differences between the summary scores for the newborns with Apgar scores 4 to 7 and newborns with Apgar scores 8 to 10 at 5 minutes after delivery.

There were three newborns with mild or moderate newborn encephalopathy and one newborn with severe newborn encephalopathy in the group with asphyxia. The mean umbilical artery buffer base value for this small subgroup was 25.4 mmol/L. There were no striking differences between the Lester summary scores for the newborns with encephalopathy in relation to those without encephalopathy. However, the subgroup with newborn encephalopathy tended to have lower motor, range of state, and autonomic regulation summary scores in the newborn behavioral assessment scale administered 2 weeks after delivery.

Comment

No reports have been found that used the newborn behavioral assessment scale to assess the association of biochemically determined intrapartum fetal asphyxia. This was a matched cohort study of mature newborns with similar birth weight and an equal number of growth retarded newborns in each group. This design controls for the different patterns of newborn behavior in the term infant in relation to the preterm newborns[12] and in the growth-retarded infant in relation to the normally grown newborns.[13]

Newborns of the group with asphyxia were derived from blood gas and acid-base screening of a large number of deliveries, which in general were representative of the obstetric population in this regional referral center. As anticipated, the Apgar scores of the group with asphyxia were significantly lower than those in the control group. However, only four newborns exhibited signs of newborn encephalopathy. Thus the fact that the study population does not reflect the severe end of the spectrum of intrapartum fetal asphyxia may have mitigated against our finding group differences.

Three days after delivery the Lester newborn behavioral assessment scale summary scores for the group with asphyxia were of the same order as those of the control group. This was also true when the newborns in the group with asphyxia with more severe metabolic acidosis were compared with those in the control group. Asphyxiated newborns with low Apgar scores at 1 and 5 minutes may represent a more severe asphyxial insult to the fetus. However, the summary scores of newborns with low Apgar scores were not significantly different from those of the newborns with good Apgar scores.

The results of the 3-day newborn assessment are in keeping with the contention that many newborns who have undergone an intrapartum asphyxial insult will not display signs of central nervous system injury. The protection of the central nervous system is presumably because of fetal cardiovascular and cerebral oxygen consumption compensatory mechanisms. Fetal hypoxemia results in increased arterial pressure as a result of increased vascular resistance associated with a redistribution of cardiac output. There is reduced blood flow to the pulmonary, renal, gastrointestinal circulation, and the body, and increased blood flow directed to the brain, heart, and adrenals.[14-16] Recent studies of fetal lamb indicate normal cerebral oxygen consumption will continue for some time in the presence of a relative degree of fetal hypoxia.[17]

Newborns with seizures have been shown to have less favorable newborn behavioral assessment scale scores.[18] Our four newborns with evidence of newborn encephalopathy had less favorable Lester summary scores at 2 weeks after delivery. Given this trend it is reasonable to assume that a larger sample of newborns with severe encephalopathy might have confirmed this finding.

The expression of abnormal newborn behavior because of a central nervous system asphyxial insult may be delayed. When the Lester summary scores for the newborn behavioral assessment scale administered 2 weeks after delivery were examined, the group with asphyxia in relation to the control group had a lower summary score for range of state. The group with asphyxia had lower scores for six individual items of the newborn behavioral assessment scale that are components of the range of state and motor summary scores. These differences are small and of limited statistical significance. However, the possibility of a delayed expression of the effect of an asphyxial insult of the central nervous system deserves further assessment.

The results of the present study suggest that the threshold of central nervous system injury as a result of intrapartum fetal asphyxia is at the severe end of the spectrum of asphyxia when it is expressed by objective measures of metabolic acidosis.

REFERENCES

1. Low JA. Fetal acid-base status and outcome. In: Hill A, Volpe JJ, eds. Fetal neurology. New York: Raven Press, 1989:195-214.
2. Freeman JM, ed. Prenatal and perinatal factors associated with brain disorders. Bethesda, Maryland: US Department of Health and Human Services, 1985; Public Health Service, NIH publication no 85-1149.
3. Low JA. The role of blood gas and acid-base assessment in the diagnosis of intrapartum fetal asphyxia. AM J OBSTET GYNECOL 1988;159:1235-40.
4. Pasamanick B, Lilienfield AM. Association of maternal and fetal factors with development of mental deficiency. T. Abnormalities in the prenatal and paranatal period. JAMA 1959;159:155-60.
5. Brazelton TB. Neonatal behavioural assessment scale. London: Spastics International Medical Publications in association with William Heinemann Medical Books, 1973.
6. Tronick E, Brazelton TB. Clinical uses of the Brazelton neonatal behavior assessment. In: Friedlander BZ, Steritt GM, Kirk GE, eds. Exceptional infant (vol 3). Assessment and intervention. New York: Brunner/Mazel, 1975:137.
7. Brazelton TB. In: Sameroff AJ, ed. Organization and stability of newborn behavior; a commentary of the Brazelton Neonatal Behavioral Assessment Scale. Chicago: Society for Research in Child Development, 1978;177:1
8. Streissguth AP, Barr HM, Martin DC. Maternal alcohol use and neonatal habituation assessed with the Brazelton scale. Child Dev 1983;54:1109-18
9. Chasnoff IJ, Burns WJ, Schnell SH, Burns KA. Cocaine use in pregnancy. N Engl J Med 1985;313:666-9.
10. Lester BM, Als H, Brazelton TB. Regional obstetrical anesthesia and newborn behaviour: a reanalysis toward synergistic effects. Child Dev 1982;53:687-92.
11. Kuhnert BR, Kennard MJ, Linn PL. Neonatal neurobehavior after epidural anesthesia for cesarean section: a comparison of bupivacaine and chloroprocaine. Anesth Analg 1988;67:74-8.
12. Als H, Lester BM, Tronick E. Toward a systematic assessment of preterm infants behaviour. In: Fitzgerald HE,

Lester BM, Yogman ME, eds. Theory and research in behavioral pediatrics. New York: Plenam Publishing Corp, 1982;1:35-132.
13. Lester BM, Garcia-Coll C, Valcarcel M, Hoffman J, Brazelton TB. Effects of atypical patterns of fetal growth in newborn (NBAS) behavior. Child Dev 1986;57:11-19.
14. Peeters LH, Sheldon RE, Jones MD, Makowski EL, Meshia G. Blood flow to fetal organs as a function of arterial oxygen content. AM J OBSTET GYNECOL 1979; 135:637-46.
15. Ashwal S, Majcher JP, Longo LD. Patterns of fetal lamb regional cerebral blood flow during and after prolonged hypoxia: studies during posthypoxic recovery period. AM J OBSTET GYNECOL 1981;139:365-72.
16. Bocking AD, Gagnon R, White SE, Homan J, Milne KM, Richardson BS. Circulatory responses to prolonged hypoxemia in fetal sheep. AM J OBSTET GYNECOL 1988; 159:1418-24.
17. Richardson BS, Rurak G, Patrick JE, Homan J, Carmichael L. Cerebral oxidative metabolism during sustained hypoxemia in fetal sheep. J Dev Physiol 1989;11:37-43.
18. Emory EK, Tynan WD, Davie R. Neurobehavioral anomalies in neonates with seizures. J Clin Exp Neuropsychol 1989;11:231-40.

Sexually transmitted diseases and human immunodeficiency virus infection among women with pelvic inflammatory disease

Bente Hoegsberg, MD,[a] Ovadia Abulafia, MD,[a] Alexander Sedlis, MD,[a] Joseph Feldman, DrPH,[b] Don DesJalais, PhD,[d] Sheldon Landesman, MD,[c] and Howard Minkoff, MD[a]

Brooklyn and New York, New York

Both human immunodeficiency virus infections and pelvic inflammatory disease are sexually acquired illnesses of great consequence to women. This study was undertaken to determine if women hospitalized with pelvic inflammatory disease, in a community endemic for human immunodeficiency virus, were at high risk to be infected with human immunodeficiency virus and if human immunodeficiency virus infections altered their hospital course. One hundred ten women hospitalized with pelvic inflammatory disease in Brooklyn (in a hospital in which 2% of parturients are human immunodeficiency virus seropositive) agreed to human immunodeficiency virus testing; 15 (13.6%) were found to be seropositive. Seropositive women were significantly more likely to have an admission white blood cell count $<10,000/mm^3$ ($p = 0.001$). Human immunodeficiency virus seropositivity was not associated with a higher frequency of other sexually transmitted diseases although there was a trend toward more cases of syphilis among human immunodeficiency virus–infected women. Similarly, although there was no significant difference in rates of operative intervention (26.6% among seropositive and 8.4% among seronegative; $p = 0.058$), there was a trend toward more surgery among those who were human immunodeficiency virus infected. Women hospitalized with pelvic inflammatory disease, in a community endemic for human immunodeficiency virus, are at high risk for human immunodeficiency virus infection. More research is needed to verify a trend toward more refractory infections among human immunodeficiency virus–infected women. (AM J OBSTET GYNECOL 1990;163:1135-9.)

Key words: Human immunodeficiency virus (HIV), pelvic inflammatory disease (PID), sexually transmitted diseases (STD)

From the Departments of Obstetrics and Gynecology,[a] Preventive Medicine,[b] and Internal Medicine,[c] State University of New York, Health Science Center at Brooklyn, and the Chemical Dependency Institute, Beth Israel Medical Center, New York.[d]
Supported by grant U62/CCU 20 1085 from the Centers for Disease Control.
Received for publication March 14, 1990; accepted May 24, 1990.
Reprint requests: Howard L. Minkoff, MD, SUNY/Health Science Center at Brooklyn, Box 24, 450 Clarkson Ave., Brooklyn, NY 11203.
6/1/22724

Pelvic inflammatory disease is a major cause of acute and chronic morbidity among women of reproductive age. Although the exact incidence of pelvic inflammatory disease is unknown, it has been estimated that by the year 2000 one of every 20 women who was 15 years old in 1970 will have had at least one episode of pelvic inflammatory disease.[1] The risk of acquiring pelvic inflammatory disease is associated with young age, increased numbers of sexual partners, and low

Table I. Clinical criteria for diagnosis of pelvic inflammatory disease

1. Abdominal direct tenderness with or without rebound
   and
2. Cervical-uterine motion tenderness
3. Adnexal tenderness plus one or more of the following
   a. Gram-negative intracellular diplococci on gram stain of endocervical smear
   b. Temperature > 38°C
   c. White blood cell count > 10,000/mm³
   d. White blood cells in intraperitoneal fluid obtained by culdocentesis or laparoscopy
   e. Pelvic abscess on bimanual examination or ultrasonography

socioeconomic status. Infections with human immunodeficiency virus (HIV) and other sexually transmitted diseases (STDs) are increasing among the same groups.[2, 3] Since these infections, as well as pelvic inflammatory disease, are sexually transmitted, it is possible that women who have pelvic inflammatory disease are at risk for HIV infection. Further, coincident infection with HIV has already been shown to influence the course of other STDs and to be associated with increases in mortality from a variety of non–"acquired immunodeficiency syndrome" infections.

The following cross-sectional study was undertaken to assess the prevalence of HIV infection among women hospitalized with pelvic inflammatory disease in a community endemic for HIV[5] and to determine if such women, who are also HIV antibody positive, have a higher prevalence of other STDs and are more severely ill.

Material and methods

Women admitted to Kings County Hospital Center between November 1987 and October 1988 who fulfilled published criteria for the diagnosis of pelvic inflammatory diseases (Table I) were eligible to participate in the study. Participants signed a study consent form, as well as a New York State consent form for HIV antibody testing. The study protocol was approved by the hospital institutional review board and the New York City Health and Hospital Corporation.

On admission diagnostic tests for STDs were performed. Saline solution washings from the posterior fornix were examined microscopically for the presence of *Trichomonas vaginalis, Candida albicans,* and bacterial vaginosis, which was diagnosed when the pH was alkaline, clue cells were present, and the "whiff" test was positive. Additionally, samples from the cervix were cultured for *Chlamydia trachomatis* according to a technique described elsewhere.[7] Specimens from the cervix, urethra, and anus were cultured for *Neisseria gonorrhoeae.* Samples from the cervix were cultured for herpes simplex virus.[7] All patients had Papanicolaou smears, which were assessed for koilocytes, multinucleation, and other changes indicating human papillomavirus infection.[8] Blood was drawn for rapid plasma reagin tests (followed, when positive, with fluorescent treponemal antibody tests) and HIV antibodies. HIV serologic testing was performed at the New York City Department of Health laboratory. Samples repeatedly positive by enzyme-linked immunosorbent assay were confirmed by Western blot.

Patients underwent detailed interviews about demographic factors, sexual practices, and drug use. A cross-sectional comparison between HIV-positive and HIV-negative subjects was performed for STDs, physical findings, and demographic characteristics.

The HIV antibody status of study subjects was not known by treating physicians. All STDs, including pelvic inflammatory disease, were treated per Centers for Disease Control guidelines.[9]

Statistical analysis. Differences between HIV-positive and HIV-negative patients were compared with Fisher's exact test. All statistical tests were two-tailed. To compensate for asymmetry and highly discrete data, a mid p value was obtained by subtracting half of the probability of the observed statistic from the exact value.[9] Odds ratios and their 95% confidence intervals were computed by exact methods with conditional maximum likelihood estimations.[10]

Results

During the 12-month study period 190 eligible patients were admitted with pelvic inflammatory disease. Of these, 144 were offered entry into the study. The remainder (n = 46) had signed out against medical advice or were otherwise unavailable (discharged before study personnel could approach them or admitted at a time when study personnel were not available). Of the 144 patients who were offered entry, 112 (78%) accepted. Two were later excluded because they had ectopic pregnancies. The majority of patients who refused to participate in the study stated (1) that they did not want to know their HIV antibody status or (2) that they had to ask their boyfriends first.

Fifteen of the 110 patients (13.6%; 95% confidence interval, 7.9% to 21.5%) who entered the study were HIV antibody positive. There was no significant difference in the mean age (28.5 ± 6.9 vs 28.1 ± 4.7 years) or parity (1.8 ± 1.3 vs 1.2 ± 1.2) between seropositive and seronegative patients. Among HIV-positive patients two were parenteral drug users, two were sex partners of parenteral drug users, and four both used parenteral drugs and had drug-using partners. Two additional patients came from countries where heterosexual spread is known to occur. Five women acknowledged no risks and had no signs of

Table II. Hospital course of HIV-antibody-positive and HIV-antibody-negative patients

	HIV status					
	Positive	*Negative*	*Total*	*p Value*	*Odds ratio*	*95% Confidence interval*
n	15	95	110			
Length of stay (days)	5.8 ± 2.0	5.6 ± 2.9		0.74		
Admission white blood cell count >10,000/mm³	6/15 (40%)	85/95 (89%)	91/100 (83%)	0.001	0.082	0.02-0.28
Admission temperature >38°C	13/15 (86%)	76/95 (80%)	89/110 (81%)	0.73	4.62	0.37-11.37
Tuboovarian abscess	4/15 (22.6%)	13/95 (13.7%)	17/110 (15.5%)	0.24	2.27	0.55-8.14
Surgery	4/15 (26.6%)	8/95 (8.4%)	12/110 (10.9%)	0.058	3.89	0.89-15.29

Table III. STDs among HIV-antibody-positive and HIV-antibody-negative patients

	HIV status					
	Positive	*Negative*	*Overall*	*p Value*	*Odds ratio*	*95% Confidence interval*
Chlamydia	15% (2/13)	16% (14/89)	16% (16/102)	0.99	0.97	0.13-4.49
Gonorrhea	33% (5/15)	25% (23/91)	27% (28/106)	0.53	1.47	0.42-4.78
Candida albicans	7% (1/14)	14.1% (12/85)	13.1% (13/99)	0.68	0.47	0.02-3.11
Human papilloma virus (Papanicolaou smear)	29% (4/14)	25% (22/87)	26% (26/101)	0.75	1.18	0.29-4.09
Herpes simplex virus (culture)	8.33% (1/12)	1.96% (1/31)	3.17% (2/63)	0.35	4.4	0.11-180.4
Trichomonas	13% (2/15)	12% (11/33)	12% (13/108)	0.99	1.15	0.16-5.32
Bacterial vaginosis	14% (2/14)	11% (10/91)	11.4% (12/105)	0.66	1.35	0.18-6.42
Positive VDRL and FTA tests	53% (8/15)	34% (31/92)	36% (39/107)	0.16	2.23	0.72-7.04
Hepatitis	7% (1/15)	2% (2/91)	3% (3/106)	0.37	3.13	0.10-43.49

VDRL, Venereal Disease Research Laboratory; *FTA*, fluorescent treponemal antibody.

intravenous drug use (needle tracks). In sum heterosexual transmission was the most likely mode of transmission in 9 of 15 (60%) patients.

On admission 40% (6/15) of the HIV-positive patients and 89% (85/95) of the HIV-seronegative patients had a white blood cell count of ≥10,000/mm³ ($p < 0.001$) (Table II). A temperature >38° C on admission was found in 86% (13/15) and 80% (76/95) of seropositive and seronegative patients, respectively ($p = 0.73$). The mean hospital stay was 5.8 days for seropositive patients and 5.6 days for seronegative patients ($p = 0.75$). Four of the 15 seropositive women (26.6%) had tuboovarian abscesses confirmed ultrasonographically, compared with 13 of 95 seronegative women (13.7%) ($p = 0.24$). Thus 4 of 17 women with tuboovarian abscesses (23.5%) were seropositive, compared with 11 of 93 women without tuboovarian abscesses (11.8%). Four of the 15 seropositive women (26.6%) had persistent fevers that were refractory to antibiotics and they required surgery: salpingo-oophorectomy ($n = 2$), total abdominal hysterectomy and bilateral salpingo-oophorectomy ($n = 1$), and laparoscopy ($n = 1$). Eight of the seronegative women (8.4%) had surgery: total abdominal hysterectomy and bilateral salpingo-oophorectomy ($n = 5$), unilateral salpingo-oophorectomy ($n = 2$), and laparoscopy ($n = 1$). The difference in the proportion of patients undergoing operation (26.6% vs 8.4%) by HIV status missed attaining statistical significance at the traditional level ($p = 0.058$; 95% confidence interval, 0.89 to 15.25).

As can be seen in Table III there was no significant difference between seropositive and seronegative patients in rates of STDs, although the difference in syphilis rates (53% vs 34%; odds ratio, 2.23; 95% confidence interval, 0.72 to 7.043) approached significance ($p = 0.16$).

Comment

Pelvic inflammatory disease, because of its prevalence, cost, and consequent morbidity, is one of the most important STDs among women. Sexual risk behaviors are clearly associated with the acquisition of both pelvic inflammatory disease and HIV. In our study of 110 in-

patients with pelvic inflammatory disease in a community that is endemic for HIV we indeed found a high rate (13.6% with a lower limit of 8%) of HIV infection, particularly among women with evidence of tuboovarian abscesses (23.5%) or white blood cell counts $<10,000/mm^3$ (47.4%). The rates are higher than previously reported from women at the same institution who were giving birth[12] or who had cervical dysplasia.[13]

HIV infections among women are being acquired increasingly through heterosexual contact. This finding was also apparent in our study. Almost two thirds of patients' putative risk for HIV infection was sexual contact. However, parenteral drug use continued to be an important risk factor with more than half of the patients either using intravenous drugs or having as their risk factor heterosexual contact with an intravenous drug user. The rate of HIV infection, even among that cohort with no intravenous drug use or sexual exposure to intravenous drug users, was sufficiently high to warrant routine counseling and offering of HIV testing to all inpatients with pelvic inflammatory disease in this community. As noted, groups at particularly high risk for HIV infection include those with a tuboovarian abscess (23.5%) and those with white blood cell counts $<10,000/mm^3$ (9/19, 47.4%).

The low white blood cell count found among women who otherwise fulfilled criteria for pelvic inflammatory disease could reflect an HIV-mediated blunting of the normal leukocytosis associated with acute pelvic inflammatory disease. HIV infections are associated with a lymphophopenia that could have contributed to this finding.

The clinical course of pelvic inflammatory disease among HIV-infected women has not been previously described. Although the in-hospital course of patients in our study did not vary in a statistically significant manner with serologic status, there was a trend toward more tuboovarian abscesses and more instances of operative intervention among seropositive women, and our study design might mask other subtle differences. If, for example, HIV-infected women have more severe cases of pelvic inflammatory disease, it would be more difficult to detect such an effect in a population consisting exclusively of inpatients. Thus, if HIV-infected women with pelvic inflammatory disease are more likely to require in-hospital care, our design would not detect that effect. Although the design and small sample size limit our ability to distinguish significant differences, HIV-seropositive women were at least twice as likely to have tuboovarian abscess and to undergo surgery. Given the possibility of more refractory pelvic inflammatory disease, it might be prudent to treat all known HIV-infected women with pelvic inflammatory disease aggressively, with consideration given to inpatient treatment for all such women.

The prevalence rate of syphilis among HIV seropositive women in this population was 53%. Although not quite statistically significantly higher than that of seronegative women in this study, the high rate of syphilis among HIV-positive patients is in concordance with that reported elsewhere.[14] Antecedent infection with syphilis or other ulcerative diseases has been reported to enhance acquisition and transmission of HIV.[15] Coincident infection with HIV can also modify the course of diseases such as syphilis. In our study population patients with pelvic inflammatory disease who were HIV seropositive did not have a significantly different pattern of associated STDs, other than syphilis, or vaginitis than that seen among HIV-seronegative patients. The rate of STDs among all patients, however, was quite high for infections ranging from human papillomavirus to hepatitis.

A potential bias in the study related to reported HIV prevalence involves ascertainment in recruitment. We do not know the characteristics of the patients who elected not to participate. However, study personnel strongly believed that many of the patients who elected not to participate were at very high risk of HIV infection because of apparent drug use and noncompliance during admission. Additionally patients who were not eligible because they signed out against medical advice before completing therapy for pelvic inflammatory disease may be at particularly high risk. It is possible therefore that the rate of HIV infection may have been understated.

In sum, women from communities that are endemic for HIV, who are treated in the hospital for pelvic inflammatory disease, represent a group at high risk for HIV infections. Further research is needed to evaluate a possible trend toward more refractory pelvic inflammatory disease among HIV-infected women.

We acknowledge Tonya Dotson's contributions to data collection.

REFERENCE

1. Curren JW. Economic consequences of pelvic inflammatory disease in the United States. AM J OBSTET GYNECOL 1980;138:848.
2. CDC. Syphilis and congenital syphilis in the United States 1987-1988. MMWR 1988;37:487-?
3. Haverkos HW, Edelman R. The epidemiology of acquired immunodeficiency among heterosexuals. JAMA 1988; 210:1922-9.
4. Johns DR, Tierney M, Felsenstein D. Alteration in the natural history of neurosyphilis by concurrent infection with the human immunodeficiency virus. N Engl J Med 1957;316:1569-72.

5. Landesman S, Minkoff H, Holman S, McCalla S, Sijih D. Serosurvey of human immunodeficiency virus in parturients. JAMA 1987;258:2701-3.
6. Hager DW, Eshenbach DA, Spence M, Sweet RL. Criteria for diagnosis and grading of salpingitis. Obstet Gynecol 1983;61:113-4.
7. Minkoff H, Grunebaum AN, Schwarz R, et al. Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. AM J OBSTET GYNECOL 1984;150:965-72.
8. Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. Acta Cytol 1978;20:505.
9. CDC. 1985 STD treatment guidelines. MMWR 1985;34:1-35.
10. Lanester HO. Significance tests in discrete distributions. J Am Stat Assoc 1961;56;223-34.
11. Rothman L. Modern epidemiology. 168-70 Little Brown, 1986.
12. Landesman S, Minkoff HL, Holman S, Mccalla S, Sijin O. Sero survey of human immunodeficiency virus infection in parturients. JAMA 1987;258:2701-3.
13. Maiman M, Fructer RG, Serur E, Boyce JG. Prevalence of human immunodeficiency virus in a colposcopy clinic. JAMA 1988;260:2214-5.
14. Stamm WE, Handsfield HH, Rompalo AM, Ashley RL, Roberts PL, Corey L. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA 1988;260:1429-33.
15. Holmberg SD, Horsburgh CR, Ward JW, Jaffe HM. Biologic factors in the sexual transmission of human immunodeficiency virus. J Infect Dis 1989;160:116-25.

The effect of large uterine fibroids on urinary bladder function and symptoms

Rami Langer, MD, Abraham Golan, MD, Menachem Neuman, MD, David Schneider, MD, Ian Bukovsky, MD, and Eliahu Caspi, MD

Zerifin, Israel

Fourteen patients with large uterine fibroids and urinary symptoms were treated with monthly injections of [D-Trp^6]-luteinizing hormone–releasing hormone microcapsules. The average uterine size before treatment was 728 ml; it dropped to 323 ml (a drop of 55%) after treatment. Urinary symptoms of diurnal frequency disappeared in 11 of 12 patients ($p < 0.005$) after the reduction of uterine size. Urgency decreased in 11 of 13 ($p < 0.005$) and nocturia in eight of 10 ($p < 0.02$). No differences were found before and after treatment in the symptoms of urge incontinence and stress incontinence in the cystometric and urethral pressure profile measurements. Urinary symptoms of frequency, urgency, and nocturia may be caused by the direct pressure exerted on the bladder by the enlarged uterus. Symptoms of urge incontinence and stress incontinence deserve a more specific treatment as they are not related to uterine size. (AM J OBSTET GYNECOL 1990;163:1139-41.)

Key words: Uterine fibroids, gonadotropin-releasing hormone analogs, bladder symptoms

Patients who have large pelvic tumors frequently have evidence of pressure on the urinary bladder giving rise to symptoms of frequency, urgency, and incontinence. These symptoms are common with large uterine fibroids, which are the most common tumors of the female pelvis. Removal of these fibroids may result in relief of the patients' urinary symptoms, as suggested by some.[1] On the other hand, surgical removal of the uterus may cause further exacerbation of the urinary symptoms because of bladder denervation.[2] Others[3] state that urinary symptoms are very rarely the result of direct uterine pressure on the bladder and, as such, urinary symptoms are a most inadvisable indication for hysterectomy.

The purpose of this study was to assess both objectively and subjectively the influence of large uterine fibroids on the urinary bladder in a model in which these tumors could be reduced in size by nonsurgical means.

From the Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, affiliated to Sackler School of Medicine, Tel Aviv University.
Received for publication March 13, 1990; revised June 1, 1990; accepted June 6, 1990.
Reprint requests: Dr. R. Langer, Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin 70300, Israel.
6/1/22992

Table I. Clinical data before and 2 months after decapeptyl treatment ($N = 14$)

	Before treatment	*After treatment*	*Significance*
Diurnal frequency	12 (85%)	1 (7%)	$p < 0.005$
Nocturia	10 (71%)	2 (14%)	$p < 0.02$
Urgency	13 (93%)	2 (14%)	$p < 0.005$
Urge incontinence	5 (35%)	4 (28%)	NS
Stress incontinence	6 (43%)	5 (35%)	NS

Table II. Cystometric data before and after 2 months of decapeptyl treatment ($N = 14$)

	Before treatment	*After treatment*
Capacity (ml)	480 ± 49.3	475 ± 50.1
Residual (ml)	22.1 ± 16.2	20.7 ± 19.3
PR_1 (mm H_2O)	10.7 ± 3.2	1.3 ± 5.4
PR_2 (mm H_2O)	4.6 ± 4.1	5.2 ± 3.8
Maximal voiding pressure (mm H_2O)	34.7 ± 9.8	36.5 ± 7.3
Peak flow rate (ml/sec)	31.8 ± 6.2	32.7 ± 4.8

PR_1, Pressure rise on filling; PR_2, pressure rise on standing.
p = NS.

Material and methods

At Assaf Harofeh Medical Centre we treat patients with large uterine fibroids scheduled for operation with gonadotropin-releasing hormone (GnRH) analogues for 2 months beforehand to reduce the uterine size; this results in less bleeding before and during operation. From these patients we selected 14 (uterine size >17 weeks of gestation) with lower urinary tract symptoms.

Uterine size was determined by ultrasonography according to the formula for ellipsoid tumors, $V = 4/3R_1 \times R_2 \times R_3 \times \text{II}$.[4] Each patient had two monthly intramuscular injections of [D-Trp^6]-luteinizing hormone–releasing hormone microcapsules (Decapeptyl, 3.2 mg; Ferring, Kiel, Federal Republic of Germany). The injections were given at 2 months and 1 month before operation. Ultrasonographic examinations were performed before the GnRH analog treatment and just before operation.

Before the first injection of the GnRH analog and again immediately before operation each patient had a full history taken and the following urinary symptoms were recorded: diurnal frequency, nocturia, urgency, urge incontinence, and stress incontinence.

The urodynamic investigation included cystometry and uroflowmetry, performed by the urinary investigation system 5000 (Lectromed, St. Peter, Jersey, Channel Islands), with saline solution used as the filling substance at room temperature and at an infusion rate of 100 ml/sec. Measurements were made with the patient in the supine and standing positions. Urethral profile pressures were performed with the dual Gaeltec microtransducer catheter (Glasgow). These tests at rest and on stress were described elsewhere.[5] Pressure transmission ratios[6] were calculated from the stress profile at four equidistant points along the functional length of the urethra. Diurnal frequency was defined as the passage of urine six or more times during the day or twice or more at night. The methods, definitions, and units conform to the standards proposed by the International Continence Society.[7] The statistical method used was McNemar's paired χ^2 test.

Table III. Urethral pressure profile before and 2 months after decapeptyl treatment ($N = 14$)

	Before treatment	*After treatment*
Absolute length (cm)	49.8 ± 5.2	47.9 ± 6.1
Functional length (cm)	44.7 ± 4.5	46.1 ± 4.1
Maximal urethral pressure	58.6 ± 10.4	57.3 ± 11.7
Maximal urethral closure pressure (mm H_2O)	52.4 ± 13.1	50.3 ± 12.8
Pressure transmission (ratio %)		
Q_1	92.8 ± 3.2	94.1 ± 2.6
Q_2	98.7 ± 6.3	99.1 ± 5.3
Q_3	99.8 ± 4.5	98.1 ± 5.1
Q_4	71.7 ± 9.8	73.1 ± 6.7

p = NS.

Results

The mean age of the patients was 44.2 years (range, 39 to 50), and mean parity was 2.6 (range, 1 to 4). Before the [D-Trp^6]-LH-RH treatment, the average uterine size, as measured by ultrasonography, was 728 ml; this dropped to 323 ml after 2 months of treatment (drop of 55%). Clinically a significant decrease (Table I) in the incidence of diurnal frequency, urgency, and nocturia was found after 2 months of [D-Trp^6]-LH-RH treatment ($p < 0.005$, <0.005, and <0.02, respectively). Symptoms of urge incontinence and stress incontinence were not affected by the decrease in uterine size.

Urodynamically, there were no significant differences between the pretreatment and posttreatment values of capacity, residual volume, and pressure rise on filling or standing, in the maximal voiding pressure, and in the peak flow rates (Table II).

Urethral pressure curves (Table III) showed no significant differences regarding values of urethral length, closure pressure, and pressure transmission ratios before and after treatment with GnRH analogs.

Comment

Treatment of uterine fibroids with GnRH analogs, their mode of action, indications, and results are well documented.[8] We treat patients with large fibroids with GnRH analogues 2 months before operation to reduce uterine size, facilitate surgery, and reduce blood loss. This reduction in uterine size (55% of the initial volume) enabled us to examine the "net" effect of the large uterus on the bladder in patients with urinary symptoms. The commonly held view that surgical removal of the enlarged uterus may cause disappearance of these urinary symptoms is controversial, mainly because hysterectomy by itself may cause urinary disturbances.[2,9] In a recent report[10] we showed that abdominal hysterectomy for benign conditions in patients without urinary symptoms does not cause urinary symptoms after operation.

In this preliminary study we proved that patients with large uterine fibroids and urinary symptoms had a significant reduction in symptoms of diurnal frequency, urgency, and nocturia merely by reducing the uterine size. One may assume that these symptoms are caused by the direct pressure exerted on the bladder by the enlarged uterus. On reduction of uterine size we found no changes in either cystometric, uroflowmetry, or urethral profile pressure measurements or in the symptoms of urge and stress incontinence. These symptoms deserve more specific treatment because they are most probably unrelated to uterine size.

REFERENCES

1. Te Linde RW, Mattingly RF. Operative gynecology. Philadelphia: JB Lippincott, 1970:149.
2. Farghaly SA, Hindmarsh JR, Worth PHL. Post hysterectomy urethral dysfunction: evaluation and management. Br J Urol 1986;58:299.
3. Turner Warwick R, Brown ADG. A urodynamic evaluation of urinary incontinence in the female and its treatment. In: Berfus LL, ed. The urologic clinics of North America. Philadelphia: WB Saunders, 1979:214.
4. Shawker RM. Monitoring response in therapy. In: Brascho DJ, Shawker TH, eds. Abdominal ultrasound in the cancer patient. New York: John Wiley, 1980:113.
5. Hilton P, Stanton SL. Urethral pressure measurement by microtransducer. The results in symptom free women and in those with genuine stress incontinence. Br J Obstet Gynaecol 1983;90:919.
6. Bhatia NN, Bergman A. Modified Burch versus Pereyra retropubic urethropexy for stress urinary incontinence. Obstet Gynecol 1985;66:225.
7. Abrams PH, Blaivus JG, Stanton SL, et al. The standardisation of terminology of lower urinary tract function. Scand J Urol Nephrol 1988;114(suppl):5.
8. Golan A, Bukovsky I, Schneider D, Ron-El R, Herman A, Caspi E. DTRP-6 Luteinizing hormone releasing hormone microcapsules in the treatment of uterine leiomyomas. Fertil Steril 1989;52:406.
9. Kikken P. Supravaginal uterine amputation versus hysterectomy with reference to subjective bladder symptoms and incontinence. Acta Obstet Gynaecol Scand 1985; 64:375.
10. Langer R, Neuman M, Ron-El R, Golan A, Bukovsky I, Caspi E. The effect of total abdominal hysterectomy on bladder function in asymptomatic women. Obstet Gynecol 1989;74:205.

The natural history of thrombocytopenia associated with preeclampsia

Vern L. Katz, MD, John M. Thorp, Jr., MD, Lynda Rozas, BA, and Watson A. Bowes, Jr., MD
Chapel Hill, North Carolina

Sixty-one of 375 women with hypertension and preeclampsia in a 48-month period had thrombocytopenia. By postpartum day 3, 90% of these women had rising platelet counts, and by day 4, 59 of 61 women had counts >100,000/mm³. The two exceptions had other pathologic conditions in addition to preeclampsia. Thrombocytopenia associated with preeclampsia should resolve by postpartum day 4. (Am J Obstet Gynecol 1990;163:1142-3.)

Key words: Thrombocytopenia, preeclampsia, platelets

Preeclampsia may be associated with a low platelet count.[1] Delivery leads to resolution of preeclampsia with removal of agents that may initiate endothelial damage and platelet consumption.[1, 2] Thus thrombocytopenia should resolve after delivery. The purpose of this study is to clarify the time course of thrombocytopenia associated with preeclampsia.

Methods

We reviewed the records of obstetric patients with diagnoses of hypertension or preeclampsia at North Carolina Memorial Hospital, a university teaching hospital, over a consecutive 48-month period. Hypertension was defined as a blood pressure of 140/90 mm Hg taken on two occasions at least 6 hours apart. Preeclampsia was defined as hypertension and proteinuria (300 mg/24 hours or two measurements of 2+ on dipstick). Severe preeclampsia was diagnosed by one of the following parameters: eclampsia, pulmonary edema, >4 gm of proteinuria in 24 hours, oliguria, or diastolic blood pressure >110 mm Hg. Thrombocytopenia was omitted as a diagnostic criteria for severe preeclampsia. The lowest platelet count in a given day was used for our assessment. A rising platelet count was defined as a platelet elevation >15% from the previous day's count.

From the Division of Maternal and Fetal Medicine, Department of Obstetrics and Gynecology, School of Medicine, University of North Carolina.
Received for publication February 21, 1990; accepted April 30, 1990.
Reprint requests: Vern L. Katz, MD, Maternal and Fetal Medicine, CB# 7570, 214 MacNider Bldg., UNC School of Medicine, Chapel Hill, NC 27599-7570.
6/1/22030

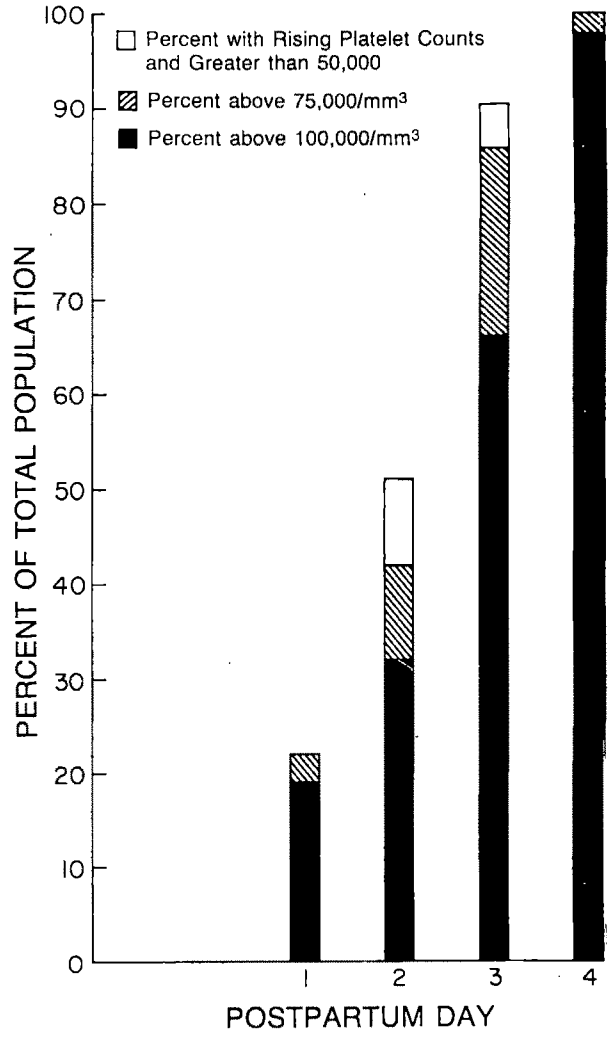


Fig. 1. Resolution of thrombocytopenia associated with preeclampsia.

Results

Three hundred seventy-five patients who were delivered during the study period were given a diagnosis of preeclampsia or hypertension. Preeclampsia was confirmed in 237 patients. Platelet counts were >150,000/mm³ in 116 women, 31 of whom had severe preeclampsia. Forty women had platelet counts between 120,000/mm³ and 150,000/mm³; nine had severe preeclampsia. Twenty patients had counts between 100,000/mm³ and 120,000/mm³, and two had severe disease. Seventeen of these 20 patients had rising platelet counts by postpartum day 2. Three patients did not have platelets measured after postpartum day 1.

Sixty-one patients with preeclampsia had platelet counts <100,000/mm³; 16 of these women had severe disease. The resolution of their thrombocytopenia is shown in Fig. 1. On postpartum day 2, 51% of women had rising platelet counts. On postpartum day 3, 90% had rising counts, and 86% of women had counts >75,000/mm³. By postpartum day 4, 59 of 61 women had rising counts and all but four women had counts >100,000/mm³. The two exceptions included a woman with chronic renal disease and normal platelet counts at delivery, but a decline to 87,000/mm³ on postpartum day 3. Her count rose to 118,000/mm³ on postpartum day 6. This patient had normal liver function tests and no evidence of hemolysis. The other patient who had no rise in platelet count had *Proteus mirabilis* sepsis, disseminated intravascular coagulopathy, and adult respiratory distress syndrome. Her platelet counts ranged between 63,000/mm³ and 96,000/mm³ on postpartum days 1 through 7.

Comment

In this series only two women did not have rising platelet counts by postpartum day 4. Both of the exceptions were atypical and had thrombocytopenia, most likely from factors other than preeclampsia. Thus if a patient with preeclampsia does not have a rising platelet count by postpartum day 3, and a count approaching 100,000/mm³ by postpartum day 4 in the absence of other pathologic conditions, she is beyond the expected time course of thrombocytopenia associated with preeclampsia.

REFERENCES

1. Weinstein L. Syndrome of hemolysis, elevated liver enzymes and low platelet counts: a severe consequence of hypertension in pregnancy. AM J OBSTET GYNECOL 1982; 142:159-67.
2. Hubel CA, Roberts M, Taylor RN, et al. Lipid peroxidation in pregnancy: new perspectives on preeclampsia. AM J OBSTET GYNECOL 1989;161:1025-34.

Continuing controversy in alloimmune thrombocytopenia: Fetal hyperimmunoglobulinemia fails to prevent thrombocytopenia

Umberto Nicolini, MD,[a] Yuen Tannirandorn, MD,[a] Pedro Gonzalez, MD,[a] Nicholas M. Fisk, MD,[a] Jeremy Beacham, MSc,[c] Elizabeth A. Letsky, MD,[a] and Charles H. Rodeck, MD[a]

London, England

Two patients with severe alloimmune thrombocytopenia were managed by weekly intrauterine platelet transfusions at 25 to 36 weeks. In one patient high-dose immunoglobulin was also administered weekly to the mother, and high maternal and fetal immunoglobulin levels were achieved. Fetal platelet counts were similar in both patients. The only variable that affected fetal platelet concentration was the posttransfusion platelet count from the previous transfusion. (AM J OBSTET GYNECOL 1990;163:1144-6.)

Key words: Alloimmune thrombocytopenia, intracranial hemorrhage, platelet transfusion

Perinatal alloimmune thrombocytopenia is associated with a perinatal mortality of 10% to 15% and severe neurologic abnormalities in up to 25% of infants, the majority of which are attributable to intracranial hemorrhage.

Ultrasonography-guided fetal blood sampling has been used in this condition to determine the platelet count and platelet antigen status at 20 to 22 weeks and again before delivery.[1] In the presence of thrombocytopenia at 37 to 38 weeks fetal intravascular platelet transfusion was performed before elective delivery. However, this strategy does not address the problem of the prevention of intracranial hemorrhage earlier in pregnancy. More recently, increases in fetal platelet counts have been reported by Bussel et al.[2] after maternally administered immunoglobulin.

We report here on two patients with severe perinatal alloimmune thrombocytopenia, managed by weekly in utero platelet transfusions; one of them also received a maternal intravenous infusion of high-dose immunoglobulin.[2]

Case reports

Two patients who had previously given birth to infants with severe perinatal alloimmune thrombocytopenia were studied. Initial fetal blood sampling was performed under ultrasonographic guidance at 26 and 25 weeks' gestation in patients A and B, respectively, by means of a 20-gauge needle. Platelet count was 28×10^9/L in patient A and 4×10^9/L in patient B.

In patient A weekly in utero transfusions of platelet antigen-1–negative concentrated platelets were performed on seven occasions. In patient B high-dose immunoglobulin (Sandoglobulin, Sandoz Pharmaceuticals, 1 gm/kg/day) was given intravenously to the mother at weekly intervals. However, after a second fetal blood sampling at 27 weeks that revealed a low platelet count, a regimen of weekly in utero transfusions of platelet antigen-1–negative concentrated platelets was adopted. The platelet antigen-1–negative donor platelet concentrations ranged from 781 to 1743 $\times$ 10^9/L and volume was 2 to 110 ml.

Serial pretransfusion and posttransfusion platelet counts for each patient are shown in Fig. 1.

Maternal and fetal blood samples were collected for determination of immunoglobulin concentration in both patients at the time of fetal transfusions (i.e., 6 days after maternal administration of immunoglobulin in patient B).

Maternal immunoglobulin levels were above the normal range on seven of eight occasions in patient B (median, 23.6 gm/L; range, 8.7 to 25.2), whereas in patient A they were always within the normal range (median, 5.1 gm/L; range, 4.4 to 7.4) (Mann-Whitney test, $p = 0.005$). Fetal immunoglobulin levels were above our normal range on all occasions in patient B after maternal administration of immunoglobulin, as opposed to normal values in patient A (Fig. 2).

Pretransfusion platelet counts correlated significantly with the posttransfusion counts from the previous transfusion (Pearson correlation coefficient, $r = 0.729$, $p = 0.007$) and were similar in patient A (median, 29.5×10^9/L; range, 18 to 44) and patient B (median, 30.0×10^9/L; range, 7 to 63) (Mann-Whitney test, $p = 0.886$).

The median rate of fall in platelet concentrations between transfusions was similar in both patients (23.6×10^9/L/day; range, 6.0 to 28.3 in Patient A and

From the Royal Postgraduate Medical School, Institute of Obstetrics and Gynecology, Queen Charlotte's and Chelsea Hospital, and the Department of Chemical Pathology, Hammersmith Hospital.
Received for publication October 6, 1989; accepted March 30, 1990.
Reprint requests: Umberto Nicolini, MD, Royal Postgraduate Medical School, Institute of Obstetrics and Gynecology, Queen Charlotte's and Chelsea Hospital, Goldhawk Road, London, United Kingdom W6OXG.
6/1/21391

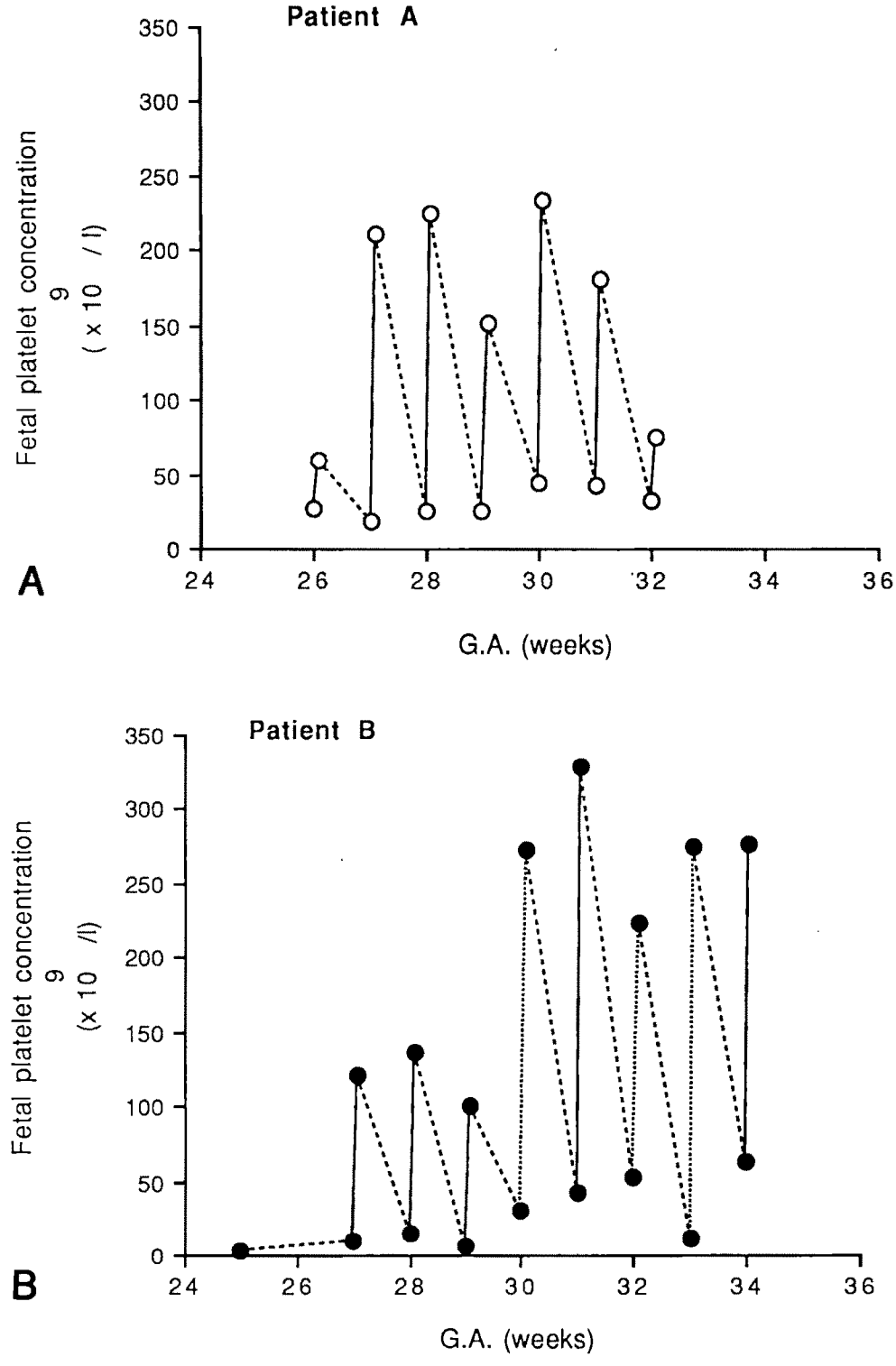


Fig. 1. Fetal platelet count before and after platelet transfusions in patient A **(A)** and in patient B **(B)**.

18.4 × 10^9/L/day; range, 10.0 to 39.4 in patient B; Mann-Whitney test, $p = 0.833$).

Neonates were delivered in good condition at 32 (case A) and 35 weeks (case B) with no signs of hemorrhage and a normal platelet count. Neither baby required postnatal platelet transfusions.

Both children are thriving and well, at ages 2 years (case A) and 6 months (case B).

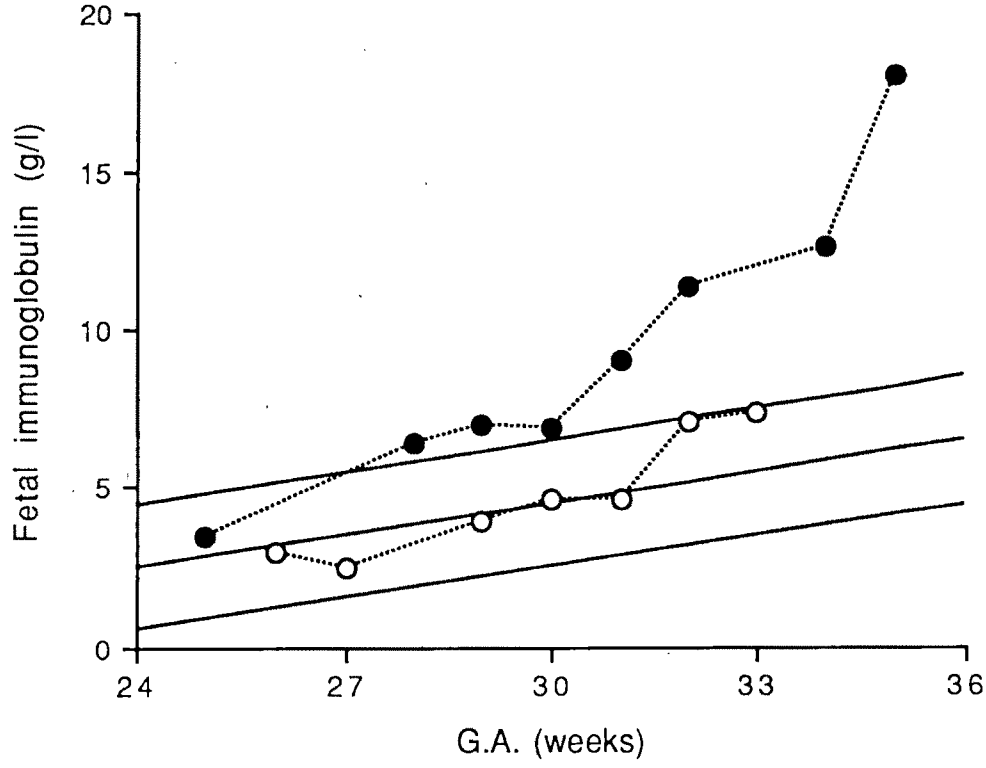


Fig. 2. Fetal immunoglobulin levels in patient A *(open circles)* and in patient B *(closed circles)* plotted against normal range for gestation.

Comment

Serial platelet transfusions appear effective in minimizing fetal thrombocytopenia in the third trimester in severe perinatal alloimmune thrombocytopenia and in preventing in utero intracranial hemorrhage. However, intrauterine transfusions carry a small procedure-related risk that by extrapolation from series of Rh-alloimmunized patients might be estimated around 2% to 4%. In this regard the recent series of seven patients reported on by Bussel et al.,[2] describing improvement in fetal platelet counts with maternally administered immunoglobulin, underlines the potential for a more conservtive treatment. However, five of seven had platelet concentrations $<100 \times 10^9$/L with two of these below 50×10^9/L. Failure of transplacental passage of immunoglobulins might explain the inconsistent results with this treatment. Indeed the immunoglobulin G levels in the fetal blood in the series of Bussel et al. were mostly within the normal range. On the contrary, transplacental passage was confirmed in our case B by high immunoglobulin concentrations in all the fetal samples, but no effect was demonstrated on platelet counts before each transfusion or in the rate of fall. This may be due to the following: (1) platelet antibodies are complement fixing and therefore Fc receptor blockade may not be adequate in preventing platelet destruction; (2) the placenta may block or alter the functional capacity of exogenous immunoglobulins.

In conclusion, in severe perinatal alloimmune thrombocytopenia adequate elevation of the fetal platelet count to prevent intracranial hemorrhage is more effectively achieved by platelet transfusions. It is not known how early in pregnancy intracranial hemorrhage can occur, although experience in some cases suggests that it may happen before 28 weeks of pregnancy. Since fetal pretransfusion platelet counts depend on the previous posttransfusion level, transfusion should aim for maximal elevation in platelet concentration.

REFERENCES

1. Kaplan C, Daffos F, Forestier F, et al. Management of alloimmune thrombocytopenia: antenatal diagnosis and in utero transfusion of maternal platelets. Blood 1988; 72:340-3.
2. Bussel JP, Berkowitz RL, McFarland JG, et al. Antenatal treatment of neonatal alloimmune thrombocytopenia. N Engl J Med 1988;319:1374-8.

Low fetal risks in pregnancies associated with idiopathic thrombocytopenic purpura

Robert F. Burrows, MD, and John G. Kelton, MD
Hamilton, Ontario, Canada

Idiopathic thrombocytopenic purpura and pregnancy are commonly associated. In this article we describe our experience in the management of 61 infants born to 50 mothers with confirmed idiopathic thrombocytopenic purpura. The focus was the neonatal cord platelet count, the parameter of greatest interest to obstetricians. None of the 61 infants had morbidity or mortality as a consequence of the thrombocytopenia. Only three of 61 infants (4.9%) had a cord platelet count that was $<50 \times 10^9$ per liter. Although 66% of the infants had a further fall in the platelet count after birth, in all the thrombocytopenia could readily be corrected. Neither maternal platelet count, maternal treatment with corticosteroids, maternal platelet-associated immunoglobulin G level, nor maternal splenectomy could be used to predict neonatal thrombocytopenia. Fetal scalp platelet sampling was likely to lead to an erroneous decision. The rareness of a poor neonatal outcome raises the question of whether obstetric interventions are justified for every pregnant patient with idiopathic thrombocytopenic purpura. (Am J Obstet Gynecol 1990;163:1147-50.)

Key words: Idiopathic thrombocytopenic purpura, pregnancy, maternal and neonatal thrombocytopenia

Idiopathic thrombocytopenic purpura is a common disease of young women. As a result, the management of idiopathic thrombocytopenic purpura during pregnancy is a clinical problem often faced by physicians. The pathophysiology of idiopathic thrombocytopenic purpura has become better understood in recent years: the thrombocytopenia is caused by an immunoglobulin G (IgG) antiplatelet autoantibody that causes platelet destruction.[1-3] Because IgG is transported across the placenta, some infants born to mothers with idiopathic thrombocytopenic purpura will be thrombocytopenic.[4,5] However, not every infant is affected and within the affected group, the degree of thrombocytopenia can vary dramatically, ranging from mild to severe. Furthermore, several years ago we noted that often the most severe thrombocytopenia does not occur until several days after delivery.[6,7] This observation has important implications for obstetricians because in some reports the recommendations concerning delivery were made on the basis of the platelet nadir. Such an approach will not produce an accurate estimate of the true hemostatic risk at the time of delivery.

From the Departments of Obstetrics and Gynecology, Medicine, and Pathology, McMaster University Medical Centre, and the Canadian Red Cross Blood Transfusion Service, Hamilton Centre.
Supported by a grant from the Medical Research Council of Canada.
Received for publication January 19, 1990; revised May 4, 1990; accepted May 18, 1990.
Reprint requests: Robert F. Burrows, MD, Department of Obstetrics and Gynecology, McMaster University Medical Centre, P.O. Box 2000, Station "A," Hamilton, Ontario, Canada.
6/1/22433

Uncertainty surrounding the infant's platelet count has led to a number of strategies to prevent or predict infant thrombocytopenia. For example, because of case reports that describe intracranial hemorrhage in thrombocytopenic neonates, many obstetricians recommend the delivery of idiopathic thrombocytopenic purpura mothers by cesarean section.[7,8] However, this approach will lead to many unnecessary cesarean sections, which themselves can result in morbidity.

The central question for the obstetrician making decisions concerning the optimal mode of delivery is the risk of severe neonatal thrombocytopenia at the time of delivery. For this reason, we analyzed our experience with idiopathic thrombocytopenic purpura in pregnancy with the focus moving from the postdelivery platelet nadir to the neonatal cord platelet count. Our reasoning was that the cord platelet count would be the best estimate of the true and immediate risk to the infant at the time of delivery. We found that the clinical impact of idiopathic thrombocytopenic purpura in pregnancy was modest: severe thrombocytopenia at delivery is distinctly uncommon. Furthermore, investigations such as fetal scalp platelet sampling are more likely to lead to unnecessary treatment than to no intervention at all.

Material and methods

The study was a retrospective analysis of all pregnant patients with idiopathic thrombocytopenic purpura delivered of infants in Hamilton, Ontario, Canada, from January 1980 to December 1989. A patient was con-

Table I. Maternal platelet count (× 10^9 per liter) and their neonates' cord platelet counts (× 10^9 per liter)

Maternal platelet count	*No.*	*Neonatal cord platelet count*			
		<50	*51-100*	*101-150*	*151+*
151+	14	2	0	0	12
101-150	21	0	1	0	20
51-100	20	1	2	1	17*
<50	5	0	0	2	3

*One set of twins.

sidered to have idiopathic thrombocytopenic purpura if she had a history of thrombocytopenia with onset before the index pregnancy. This definition was used to ensure that we did not include patients with incidental thrombocytopenia of pregnancy, a condition frequently confused with idiopathic thrombocytopenic purpura and one that does not impact on the mother or her infant.[9] The clinical diagnosis of idiopathic thrombocytopenia was made on the basis of the following criteria: (1) isolated thrombocytopenia without another hematological abnormality; (2) no secondary cause for the thrombocytopenia (hypersplenism, lymphadenopathy, systemic lupus erythematosus); and (3) a negative history for the ingestion of medication that could cause thrombocytopenia. Hamilton has three hospitals in which approximately 6000 deliveries are performed per year. All medical records from the three hospitals coded for thrombocytopenia and pregnancy were reviewed to identify those pregnancies that were complicated by idiopathic thrombocytopenic purpura.

On the basis of an analysis of the idiopathic thrombocytopenic purpura literature, we reviewed our experience with the following potential predictors of neonatal risk: maternal platelet count, maternal platelet associated IgG level, previous maternal splenectomy, steroid therapy, and fetal scalp sampling. Platelet count determinations were performed on an ethylenediamine tetraacetic acid anticoagulated specimen with a Coulter Counter (Coulter Electronics, Hialeah, Fla.). The mother or infant was said to have thrombocytopenia if the platelet counts were <150 × 10^9 per liter. Maternal platelet-associated IgG was quantitated with an immunoradiometric assay.[10] A platelet associated IgG level >5.5 fg IgG per platelet was considered abnormal.

Performance of scalp sampling. The fetal scalp blood sampling for measurement of the fetal platelet count was performed with the technique and modifications that we have described.[11] In brief, after therapeutic or spontaneous rupture of the membranes the fetal scalp was dried and a small nick was made in the scalp with the sterile razor included in the fetal blood gas kit. An ethylenediamine tetraacetic acid anticoagulant capillary sample was collected and the platelet count was determined on this sample with an automated particle counter, with the result confirmed by phase microscopy.

Results

During the study period, 50 patients with idiopathic thrombocytopenic purpura were delivered of infants in our hospitals. These 50 women had 60 pregnancies and were delivered of 61 infants.

Maternal outcome. As shown in Table I, 76.7% of mothers had thrombocytopenia at delivery and 41.7% had a platelet count <100 × 10^9 per liter. There was no serious morbidity in any of these women. Sixteen of the patients (26.7%) were receiving corticosteroids at the end of the pregnancy. No other therapy was given. Nine of the patients (18.0%) had a previous splenectomy. These women with a history of previous splenectomy were delivered of a total of 13 infants. In the entire group the cesarean section rate was 35.0% (21 of 60).

Infant outcome. The 61 infants had cord platelet counts that ranged from 24 to 420 × 10^9 per liter. A total of 52 infants (85.2%) had a cord platelet count >150 × 10^9 per liter. Three (4.9%) had a cord platelet count between 101 and 150 × 10^9 per liter. Three infants (4.9%) had a cord platelet count between 51 and 100 × 10^9 per liter and three (4.9%) had a cord platelet count <50 × 10^9 per liter (Table I). No infant had serious bleeding.

For 21 infants (34.4%) the cord platelet count (day 0) was the lowest platelet count that occurred, whereas in 40 (65.6%) of the neonates there was a further fall in the platelet count after birth. A total of 33 (54.1%) experienced their platelet nadir by day 1 or 2. Because of a further fall in platelet count after delivery, five infants required intravenous IgG. There was no evidence of serious morbidity in any infant. The neonatal platelet count had stabilized or had begun to rise in all infants by day 7.

Four of the infants with cord platelet counts <150 × 10^9 per liter were delivered by cesarean section and five were vaginally delivered. Three infants had cord platelet counts <50 × 10^9 per liter; two were vaginally delivered and the other was delivered by cesarean section because of obstetric reasons (primigravida breech presentation). None of the three had bleeding.

Prediction of infant thrombocytopenia. There was no test or maneuver that could predict neonatal thrombocytopenia. There was no correlation between the maternal platelet count and the cord platelet count at delivery (Table I). The neonatal cord platelet count was $<150 \times 10^9$ per liter in six of 26 infants (23.1%) whose mothers had a platelet count of $<100 \times 10^9$ per liter (sensitivity, 66.7%; specificity, 61.5%; positive predictive value 23.1%; and negative predictive value, 91.4%). The risk of more severe thrombocytopenia in infants, defined as a cord platelet count $<50 \times 10^9$ per liter was similar for those infants of mothers whose platelet count was $>100 \times 10^9$ per liter, compared with those mothers whose platelet counts were $<100 \times 10^9$ per liter (5.7% and 4%, respectively).

The level of platelet-associated IgG was measured in 46 pregnancies. Eight mothers had elevated platelet associated IgG, but infant cord thrombocytopenia occurred in only two infants, giving a sensitivity of 22.2%, a specificity of 83.8%, a positive predictive value of 25%, and a negative predictive value of 81.6%.

The risk of thrombocytopenia for the infants born to mothers treated with corticosteroids was the same as the risk for the infants born to mothers not treated with corticosteroids, 25% (95% CI, 3.8% to 46.2%), compared with 11.1% (95% CI, 1.9% to 20.3%).

One (7.7%) of the infants born to mothers who had undergone splenectomy had thrombocytopenia.

Fetal scalp sampling was attempted in 18 patients. In six patients the scalp sample was successful, the fetal platelet count was found to be $>50 \times 10^9$ per liter and the correct decision was made to proceed with vaginal delivery. In eight patients the sample was unsuccessful in that sufficient amounts of scalp blood for testing was not obtained or the sample was diluted by amniotic fluid and erroneously low results resulted. In these eight pregnancies a spontaneous vaginal delivery occurred before a cesarean section could be performed. All these infants had a platelet count $>50 \times 10^9$ per liter. In four infants the scalp sample produced erroneously low results that led to the wrong decision—cesarean section. In all these infants the cord platelet count was $>50 \times 10^9$ per liter.

Comment

Idiopathic thrombocytopenic purpura is an illness of young women. Some of these patients will become pregnant during an active stage of their illness. The management of the mother is simplified because the clinician can monitor the maternal platelet count and look for clinical signs of hemostatic impairment. The management of the fetus is more difficult. There is controversy within the medical community about the risk to the fetus and the type and necessity of intervention to ensure well-being. The general assumption has been that infants born to mothers with idiopathic thrombocytopenic purpura have a high risk of thrombocytopenia. Case reports that describe the occurrence of intracranial hemorrhage in these infants after delivery have led some obstetricians to recommend delivery by cesarean section in the hopes of lowering infant morbidity and mortality.[7, 8] However, the central issues are the frequency of serious morbidity in these infants and whether cesarean section could reduce the morbidity in an affected infant. The current study was performed to try to analyze predictors of neonatal thrombocytopenia.

When assessing the risk for the neonate it is important to relate the results of a diagnostic maneuver or intervention to the fetal cord platelet count. The reason is that the bleeding risk at the time of delivery is related to the fetal platelet count at that time (i.e., the fetal cord sample). We previously showed that in many of these infants the platelet count continues to fall after delivery.[6] But in some reports the platelet nadir was used as the end point in the assessment of the thrombocytopenic risk. If the thrombocytopenia is most severe several days after delivery, then an excessively pessimistic estimate of the fetal risk at the time of delivery will result.

For these reasons our study focused on the neonatal cord platelet count, the measure of greatest interest to obstetricians. We found that the risk of neonatal thrombocytopenia, defined as a cord platelet count $<150 \times 10^9$ per liter was much lower than has previously been suggested and occurred in only 14.8% of our patients (95% CI, 5.9% to 23.7%). It should be noted that in many neonates the platelet count continued to fall after delivery and in some infants treatment with intravenous IgG was required. In our study, no infant bled, and only three of the 61 infants had cord platelet counts $<50 \times 10^9$ per liter at the time of delivery. One of these three infants was delivered by cesarean section for obstetric reasons and the others were vaginally delivered. No untoward event occurred in any infant.

The reason the outcome of our infants was better than that noted by other groups remains uncertain. We were careful to include every pregnant patient with idiopathic thrombocytopenic purpura who was first seen in our region to avoid a negative reporting bias. Indeed, because of the author's interest in idiopathic thrombocytopenic purpura, it is possible that we would be referred more severe cases, making it unlikely that we would underestimate the true risk. Similarly, we were careful to exclude any patients with the benign condition, incidental thrombocytopenia of pregnancy.[9] Only those patients with true idiopathic thrombocytopenic purpura and a previous history of active disease were included in the study. It is also unlikely that the relative mildness of the neonatal thrombocytopenia

that we observed was a result of maternal treatment with corticosteroids. Only 26.7% of our mothers were treated with corticosteroids and there was no difference in the proportion of the infants with thrombocytopenia in this group compared with the nontreated group.

The low frequency of thrombocytopenia in our infants and its lack of morbidity explains why none of our predelivery diagnostic or therapeutic maneuvers were shown to be useful. We did not find a correlation between the maternal platelet count and severity of infant thrombocytopenia. Neither was a history of previous maternal splenectomy more likley to be associated with infant thrombocytopenia. Corticosteroid administration did not have an obvious beneficial affect on the infants in our series, but we cannot make conclusions concerning the usefulness of corticosteroid therapy because this was not a randomized study and it is possible that the mothers with more severe disease would be more likely to receive corticosteroids. Finally, given the low frequency of thrombocytopenia overall, it would be methodologically difficult to demonstrate efficacy of corticosteroid therapy in any study. Previously, our group reported that elevated platelet associated IgG test results in the mother were predictive of neonatal thrombocytopenia,[6] and normal platelet associated IgG levels were associated with a low possibility of neonatal thrombocytopenia. In the current study, the negative predictive value of elevated platelet associated IgG remains high, but the positive predictive value has fallen concurrent with the low prevalence of neonatal thrombocytopenia.

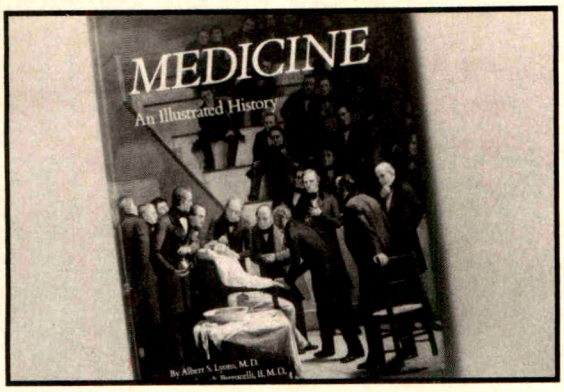
At our center we no longer perform fetal scalp platelet sampling. In our hands the test frequently fails because of technical reasons and generates a falsely low platelet estimate, which can lead to an inappropriate intervention such as cesarean section. Other physicians also have experienced problems with fetal scalp sampling.[12-15] Although we did not perform cordocentesis, we believe it is difficult to justify this procedure for two reasons. First, it has a low, but real morbidity for mother and fetus[16] and second, as demonstrated, the likelihood of benefit being achieved is also low because the risk to the infant is low.

Our article should not be interpreted to indicate that idiopathic thrombocytopenic purpura in pregnancy does not carry any risk for neonates. However, we have found that the perinatal risk and the severity of the thrombocytopenia at the time of delivery is far lower than had previously been thought. Nonetheless, because many of these infants will have a progressive fall in platelet count after delivery they should be monitored and appropriate intervention, such as intravenous IgG or corticosteroid therapy, instituted if required.

What our study does indicate is that it is uncommon for infants to be severely affected by the maternal illness at the time of delivery. The uncommonness of a poor neonatal outcome indicates that intervention associated with maternal or fetal morbidity including cordocentesis, fetal scalp sampling, and delivery by cesarean section cannot be justified for every pregnant patient with idiopathic thrombocytopenic purpura.

REFERENCES

1. McMillan R, Tani P, Mason D. The demonstration of antibody binding to platelet-associated antigens in patients with immune thrombocytopenic purpura. Blood 1980; 56:993-5.
2. McMillan R. Chronic idiopathic thrombocytopenic purpura. N Engl J Med 1981;304:1135-47.
3. Karpatkin S. Autoimmune thrombocytopenic purpura. Blood 1980;56:329-43.
4. Cines DB, Dusak B, Tomaski A, et al. Immune thrombocytopenic purpura and pregnancy. N Engl J Med 1982;306:826-31.
5. Kernoff LM, Malan E, Gunston K. Neonatal thrombocytopenia complicating autoimmune thrombocytopenia in pregnancy. Ann Intern Med 1979;90:55-6.
6. Kelton JG, Inwood MJ, Barr RM, et al. The prenatal prediction of thrombocytopenia in infants of mothers with clinically diagnosed immune thrombocytopenia. Am J Obstet Gynecol 1982;144:449-54.
7. Murray JM, Harris RE. The management of the pregnant patient with idiopathic thrombocytopenic purpura. Am J Obstet Gynecol 1976;126:449-51.
8. Kitzmiller JL. Autoimmune disorders: maternal, fetal, and neonatal risks. Clin Obstet Gynecol 1978;21:385-96.
9. Burrows RF, Kelton JG. Incidentally detected thrombocytopenia in healthy mothers and their infants. N Engl J Med 1988;319:142-5.
10. Kelton JG, Denomme G, Walker C, Horsewood P. The measurement of platelet-associated IgG using an immunoradiometric assay. J Immunoassay 1983;4:65-82.
11. Kelton JG. Management of the pregnant patient with idiopathic thrombocytopenic purpura. Ann Int Med 1983; 99:796-800.
12. Wahbeh CJ, Eden RD, Killam AP, Gall SA. Pregnancy and immune thrombocytopenia. Am J Obstet Gynecol 1984;149:238-9.
13. Christiaens GCML, Helmerhorst FM. Validity of intrapartum diagnosis of fetal thrombocytopenia. Am J Obstet Gynecol 1987;157:864-5.
14. Tchernia G, Dreyfus M, Laurian Y, Derycke M, Mirica C, Kerbrat G. Management of immune thrombocytopenia in pregnancy: response of infusions of immunoglobulins. Am J Obstet Gynecol 1984;148:225-6.
15. Tchernia G. Immune thrombocytopenic purpura and pregnancy. Curr Stud Hematol Blood Transfus 1988; 55:81-9.
16. Moise KJ, Carpenter RJ, Cotton DB, Wasserstrum N, Kirshon B, Cano L. Percutaneous umbilical cord blood sampling in the evaluation of fetal platelet counts in pregnant patients with autoimmune thrombocytopenic purpura. Obstet Gynecol 1988;72:346-50.



MEDICINE: An Illustrated History

By Albert S. Lyons, M.D., F.A.C.S. and R. Joseph Petrucelli II, M.D.; with 14 contributors

This splendid survey will provide unending hours of pleasure. You'll enjoy relaxing with it after long hours in the office.

- brilliant color pictures review every dimension of medical history
- historical breakthroughs in medicine recorded with spellbinding prose
- timeless classic makes a splendid gift

1974. 618 pages, 1,020 illustrations including 250 in full color (Book Code: 03374) $42.95 (U.S.); $56.00 (Can.)

Save time! Call toll-free: 800-426-4545, Monday-Friday, 7AM-7PM, Central Time FAX orders, 314-432-1380 Attn: Direct Marketing Please mention this number when calling: FLR-619-999-03 In Canada, call collect: 416-298-2465 For Customer Service: 800-633-6699 Monday-Friday, 7AM-6PM, Central Time

Mosby-Year Book • 11830 Westline Industrial Drive • St. Louis, MO 63146

Mosby-Year Book, Ltd. • 5240 Finch Avenue East • Scarborough, Ontario • Canada M1S 5A2

YES! Please send ____ copies of (03374) Lyons, MEDICINE: An Illustrated History, priced at $42.95 (U.S.); $56.00 (Can.), on no-risk, 30-day approval.

SAVE MONEY! Send a check with your order or charge it to your credit card. Mosby-Year Book pays the postage on all prepaid orders. Please add $3.50 per order to cover handling charges. Other orders will be billed for postage and handling.

☐ Payment enclosed

☐ Bill me, plus postage and handling

Charge my: ☐ MasterCard ☐ VISA ☐ AmEx

Card # _____ Exp. date _____

Signature _____

Business phone (_____) _____

Name _____

Address _____

City _____

State _____ Zip _____

If using a purchase order, please attach this coupon and send to Pat Newman at the Mosby-Year Book address. Outside the U.S. and Canada, please pay by check or money order in dollar equivalent. 30-day approval good in U.S. and Canada. Prices subject to change without notice. Add applicable sales tax.

FLR-619-999-03

Mosby Year Book

It's 8:00 a.m.

DO YOU KNOW WHO YOUR PROBLEM PATIENTS ARE?

MONDAY, OCTOBER 9, 1990:

Tammy McDonald is 18 weeks pregnant and has an elevated AFP. She missed her perinatal exam Friday. ***Will your office call her this morning?***

Sheena Brandt's EDD was October 2nd. You haven't heard from her. ***Will your staff follow up today?***

Brenda Martin's chart was in Dr. Jessup's office. He saw her for her last exam while you were in Delivery. ***Does anyone know where her chart is now?***

You accidentally charted the wrong EDD for Nicole Arevalo. ***Will you immediately be alerted to the error?***

If your office isn't managing high-risk situations, then you're practicing high-risk medicine. Pro-active patient tracking procedures are the only sure way to protect both your patient... and your practice.

With **The OB Manager,** you can track patient progress before negative consequences result and alert your staff to critical follow through procedures. No more lost memos. No loose details. Just common sense software that retains crucial patient data and directs your staff with daily online instructions that keep you in charge.

The OB Manager's pro-active patient care program keeps you on top of your practice.

With a full range of instant reports from patient listings by provider, status, and risk factors to past due dates and abnormal test dates, **The OB Manager provides quality assurance and protection for your future.**

OB manager

For more information contact:

THC
8669 West Camden Drive • Elk Grove, CA 95624
(916) 685-5190

The New Corometrics Model 116 Intrapartum Fetal Monitor:

Providing Advanced Capabilities to Simplify Patient Care



Building and maintaining leadership in obstetrical instrumentation is a part of the Corometrics' tradition. And Corometrics continues to focus on patient care while answering the needs of clinicians. The Corometrics' Model 116 Intrapartum Fetal Monitor is another example of this tradition.

This advanced monitor retains all the highly successful features of the Model 115, such as autocorrelation signal processing and electronic data entry. In addition, the system offers a number of new, significant monitoring capabilities.



Dual Ultrasound Adds to Your Monitoring Capabilities

The Model 116 provides dual ultrasonic heart rate monitoring, as a standard feature, for the simultaneous, external monitoring of twins. This mode is particularly useful for monitoring twins in the early stages of labor or when performing antepartum testing. Internal/ external monitoring is also provided.



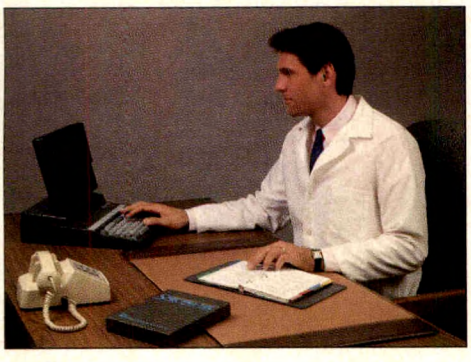
Interface With Blood Pressure Monitors For Automatic Documentation

The Model 116 accepts and automatically documents data from most widely used non-invasive blood pressure monitors, including the new Corometrics' Model 555. The measured systolic, diastolic and mean blood pressure values as well as the pulse rate are clearly recorded on the center margin of the strip chart. The Model 116 also records the time of the reading.



Beltless Tocotransducer Ensures More Complete Information

Corometrics' unique new beltless tocotransducer eliminates the instability and inconvenience associated with belted systems. A disposable adhesive baseplate holds the transducer securely in place, and the system provides reliable, useful readings even during patient position changes, sitting or walking. Patients enjoy freedom from the constrictive belt. Nurses appreciate how little attention is required to obtain a clear, continuous reading.



Data Storage and Telecommunication Improve Access to Information

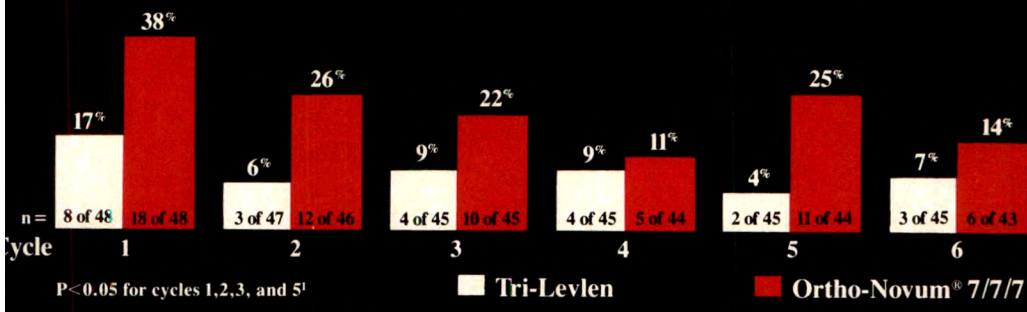
Corometrics' Model 116 stores over an hour of heart rate and uterine activity data which can be transmitted via telephone lines for review or referrals. The clinician can monitor this patient data, using Corometrics' SPECTRA-TEL® software package, in the office or at another remote location.

For more information or a demonstration call: (203) 265-5631 in CT (800) 243-3952 Extension 465

COROMETRICS MEDICAL SYSTEMS, INC.

Tri-Levlen® means a low incidence of breakthrough bleeding/spotting*†

Multicenter study demonstrates significantly less breakthrough bleeding/spotting through the first six cycles[1]



P<0.05 for cycles 1,2,3, and 5[1]

Tri-Levlen Ortho-Novum® 7/7/7

n = number of women with a bleeding event occurring during a cycle.

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

may mean a lot to a woman

* Incidence defined as percentage of women with a bleeding event occurring at least once during a pill cycle.
Serious as well as minor side effects have been reported with the use of oral contraceptives.
The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

† Defined as intermenstrual bleeding.

TRI-LEVLEN® 28 Levonorgestrel and ethinyl estradiol
TRI-LEVLEN® 21 tablets–Triphasic regimen

BRIEF SUMMARY
Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.
CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.
MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.
RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000); the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.
PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.
2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.
4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.
5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If t patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missi period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no preci information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrog combinations to induce withdrawal bleeding should not be used as a test of pregnancy.
6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, increased risk was apparent between 6 and 12 months of use.
7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tr Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lip metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of thes findings remains to be defined.
8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertensic may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in use and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposur and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated wit the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to norm after discontinuing the drug.
9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent severe, requires discontinuation of OCs and evaluation of the cause.
10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule o pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessar since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive method Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.
11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.
12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity an quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mother receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should b deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatmen and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 yea without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed an the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs shoul stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4 OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patient with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac o renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice whil receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroi hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism whic may result in a relative pyridoxine deficiency. The clinical significance of this is yet to b determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnan woman is predisposed to the development of folate deficiency and the incidence of folat deficiency increases with increasing gestation, it is possible that if a woman become pregnant shortly after stopping OCs, she may have a greater chance of developing folat deficiency and complications attributed to this deficiency. 9. The pathologist should b advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine and liver-function tests and blood components may be affected by estrogen-containin OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.
Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.
Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."
Nursing Mothers—See "Warnings."
Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.
There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.
The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.
The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.
Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.
Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.
For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

Reference: 1. Data on file, Berlex Laboratories, Inc.

TRI·LEVLEN®
Levonorgestrel and ethinyl estradiol tablets–
Triphasic regimen

BERLEX®
Dedicated to innovation in OB/GYN medicine

BERLEX Laboratories, Inc., Wayne, New Jersey 07470

© 1990, Berlex Laboratories, Inc. All rights reserved.
430-200

Laparoscopic ventrosuspensions

A review of 72 cases

Ann F. E. Yoong, MB, ChB
Merseyside, England

Laparoscopic ventrosuspension is simple to perform after diagnostic laparoscopy. Serious postoperative complication is unlikely. However, patient follow-up over 6 months has not confirmed the usefulness of laparoscopic ventrosuspension in the management of deep dyspareunia or pelvic pain in association with a retroverted uterus. The success rate of laparoscopic ventrosuspension at 6 months varies from 18.6% to 46.5%. The prior use of a Hodge pessary does not predict the success of laparoscopic ventrosuspension. (AM J OBSTET GYNECOL 1990;163:1151-3.)

Key words: Laparoscopic ventrosuspension, retroverted uterus, deep dyspareunia, chronic pelvic pain, Hodge pessary

Laparoscopic ventrosuspension has been suggested as very effective in the relief of deep dyspareunia associated with retroversion of the uterus.[1] This study analyzes the incidence and outcome of laparoscopic ventrosuspensions performed at a district general hospital in the United Kingdom over a period of 5 years.

Patients and methods

The case reports of all patients who had laparoscopic ventrosuspension at Arrowe Park Hospital, Wirral, during the period from April 1982 to October 1987 were studied. The indication for the laparoscopic ventrosuspension, the previous use of a Hodge pessary, the presence of pelvic pathologic conditions, and any postoperative complications were noted.

The "success" of the laparoscopic ventrosuspension was determined by symptomatic improvement at follow-up visit 6 weeks later and at 6-month review. The review period was extended to 2 years so that further management could be observed.

Surgical technique

All laparoscopic ventrosuspensions were performed in the manner described by Steptoe and Frangenhein.[1] A laparoscope was inserted after the creation of a carbon dioxide pneumoperitoneum. The pelvis was inspected carefully and any abnormalities were noted. Skin crease incision approximately 3 cm long were made above and parallel to each inguinal ligament. A Spencer Wells forceps was then pushed into the abdomen under laparoscopic visualization and used to grasp the round ligament at its midpoint. Each round ligament was brought out of the abdominal cavity in turn. The tension on each round ligament was adjusted to obtain the correct degree of uterine anteversion and each round ligament then was plicated to the anterior rectus sheath with Dexon or nylon sutures.

From Arrowe Park Hospital.
Received for publication April 9, 1990; accepted May 18, 1990.
Reprint requests: Ann F. E. Yoong, MD, The Women's Hospital, Liverpool, L8 7NJ, United Kingdom.
6/1/22482

Results

There were 72 laparoscopic ventrosuspensions performed in the 5 years under review. The average age of patients was 31.8 years; the majority was multiparous (Table I).

The indications for laparoscopic ventrosuspensions are shown in Table II. The primary indication for laparoscopic ventrosuspension was deep dyspareunia with chronic pelvic pain. In 43 patients (59.7%) no pelvic pathologic condition was identified at laparoscopy. Other pelfic pathologic conditions found at laparoscopy are listed in Table III.

At the 6-week follow-up 22 of the 43 patients (51.2%) in whom no pelvic condition was found stated that they felt better, 16 women (37.2%) did not feel better, and five women (27.9%) were lost to follow-up. Six months after laparoscopic ventrosuspension eight women (18.6%) reported that they felt better, 23 of the 43 (53.5%) felt they were no better, and 12 women (27.9%) failed to return (Table IV).

In 17 patients a Hodge pessary had been used to antevert the uterus before operation. These patients were listed for laparoscopic ventrosuspension only if the Hodge pessary had improved their symptoms. Table V shows that six of the 17 patients (35.3%) had improvement in their condition by laparoscopic ventrosuspension at the 6-week visit and seven of the 17

Table I. Age and parity of 72 patients

	Mean	*Range*
Age (yr)	31.8	24-42
Parity	1.7	0-4

Table II. Indication for laparoscopic ventrosuspension

Indication	*No.*	*%*
Deep dyspareunia and chronic pelvic pain	41	56.9
Deep dyspareunia	19	26.4
Chronic pelvic pain	12	16.7

Table III. Laparoscopic ventrosuspension

Pathologic findings	*No.*
No abnormality	43
Ovarian cyst	3
Pelvic adhesions	13
Endometriosis	10
Fibroids	10
Hydrosalpinx	1

patients (41.2%) were no better. At 6-months no patient felt better, seven patients (41.2%) did not feel better, and 10 patients (58.8%) were lost to follow-up.

Two years after laparoscopic ventrosuspension, 25 of the 43 patients (58.1%) were lost to follow-up. Twelve of the 43 (27.9%) had had a hysterectomy performed. Six patients (14.0%) who had had no further surgical therapy were still under review (Table VI).

Of the 72 patients who underwent laparoscopic ventrosuspension, 64 patients were seen 6 weeks after operation and eight patients did not return for follow-up. Table VII shows that there were no postoperative complications in 39 of the 64 patients (60.9%), whereas 19 women (29.7%) experienced persistent groin pain after operation and six women had wound infection (9.4%).

Comment

This is the first study that reviews laparoscopic ventrosuspension in gynecologic practice.

Laparoscopic ventrosuspension combines diagnostic laparoscopy with a simple form of therapy. In the study by Chamberlain and Brown[2] laparoscopic ventrosuspensions were performed in 0.8% of all laparscopies, primarily at the time of diagnostic laparoscopy. Candy[8] reported on the succes of a laparoscopic ventrosuspension performed for deep dyspareunia as a result of uterine retroversion. A Hodge pessary had eliminated the patient's dyspareunia previously. At her postoperative visit the patient suffered no dyspareunia.

Table IV. Follow-up of 43 patients in whom no pelvic pathologic condition was found

	6 wk		*6 mo*	
	No.	*%*	*No.*	*%*
Improved	22	51.2	8	18.6
No better	16	37.2	23	53.5
Lost to follow-up	5	11.6	12	27.9

Table V. Follow-up of 17 patients after a trial of Hodge pessary

	6 wk		*6 mo*	
	No.	*%*	*No.*	*%*
Improved	6	35.3	0	0
No better	7	41.2	7	41.2
Lost to follow-up	4	23.5	10	58.8

Table VI. Results of laparoscopic ventrosuspension in the 43 patients with no obvious pelvic pathologic conditions at 2 years

	No.	*%*
Lost to follow-up	25	58.1
Hysterectomy	12	27.9
Follow-up with no further operation	6	14.0

Table VII. Postoperative complications

Complications	*No.*	*%*
No complication	39	60.9
Groin pains	19	29.7
Wound infection	6	9.4

In this study laparoscopic ventrosuspensions were performed for chronic pelvic pain as well as deep dyspareunia. These symptoms may form part of the "pelvic pain syndrome"[4, 5] and the "chronic pelvic pain without obvious pathology."[6, 7]

In this study there were 59.7% cases in which no pelvic pathologic condition was found at laparoscopy. Gillebrand[8] reported that 63% of his patients with pelvic pain had no obvious condition at laparoscopy. Kresch et al.[9] identified 83% patients with no pelvic pathologic conditions when the pelvic pain had been in the same location for 6 or more months.

The initial improvement after laparoscopic ventrosuspension was unremarkable. Twenty-two of the 43 women (51.5%) with no obvious cause for their deep dyspareunia or pelvic pain felt better 6 weeks after the operation, whereas 16 of the 43 (37.2%) did not.

At 6 months there was a high loss to follow-up and

12 patients (27.9%) failed to return. It could be argued that those who failed to return were satisfied with their treatment; if this is assumed, 20 of the 43 patients (46.5%) were satisfied 6 months after laparoscopic ventrosuspension. Conversely, it may be argued that those who failed to returned were dissatisfied; then only eight patients (18.6%) of this group had improvement as a result of the procedure. Thus it can be seen that the success rate 6 months after laparoscopic ventrosuspension varies from 18.6% to 46.5%. The decline in patient's symptomatic improvement with time confirms Renaer's suggestion[6,7] that such patients should be followed up over a period of 1 to 2 years.

It is often stated that the success of laparoscopic ventrosuspension can be predicted from a previous trial of Hodge pessary. None of the 17 patients who had had a previously successful trial of Hodge pessary felt better at the 6-month review. However, many did not return for follow-up. If it is assumed that all 10 patients lost to follow-up at 6 months were satisfied by their treatment, then there were 10 successes among 17 patients (58.8%). If the converse is assumed, all 10 women lost to follow-up were unhappy with their treatment and no successes could be attributed to laparoscopic ventrosuspension. It is clear from this study that prior improvement with a Hodge pessary is not necessarily a good indicator of success of laparoscopic ventrosuspension.

It is interesting to note that 27.9% of these 43 patients with no obvious pelvic pathologic condition had a hysterectomy within 2 years of the laparoscopic ventrosuspension.

No serious postoperative complications occurred after laparoscopic ventrosuspension in this study. Persisting groin pain following laparoscopic ventrosuspension has been explained as a result of overtightening of the round ligaments in the course of anteversion.[10] This occurred in 29.7% of patients in this study. Urinary retention as reported by Lose and Lindholm[11] was not noted.

I thank the consultant staff at Arrowe Park Hospital for their permission to review their patients' notes.

REFERENCES

1. Steptoe PC, Frangenhein H. Laparoscopy in gynaecology. Baltimore: Williams & Wilkins, 1967:78-80.
2. Chamberlain G, Brown JC. Gynaecological laparoscopy. The report of the working party of the confidential enquiry into gynaecological laparoscopy. London: Royal College of Obstetricians and Gynaecologists, 1978:45.
3. Candy JW. Modified Gilliam uterine suspension using laparoscopic ventrosuspension. Obstet Gynecol 1976;47: 242-3.
4. Beard RW, Reginald PW, Wadsworth J. Clinical features of women with chronic lower abdominal pain and pelvic congestion. Br J Obstet Gynaecol 1988;95:153-61.
5. Beard RW, Reginald PW, Pearce S. Pelvic pain in women. Br Med J 1986;293:1160-2.
6. Renaer M. Chronic pelvic pain without obvious pathology in women. Eur J Obstet Gynecol Reprod Biol 1980;10: 415-63.
7. Renaer M. Chronic pelvic pain without obvious pathology in chronic pelvic pain in women. New York: Springer-Verlag, 1981:162-75.
8. Gillebrand PN. Investigation of pelvic pain. Communication at the scientific meeting on chronic pain—a gynaecologic headache. London: Royal College of Obstetricians and Gynaecologists, 1981.
9. Kresch JK, Seifer D, Sachs LB, Barrese L. Laparoscopy in 100 women with chronic pelvic pain. Obstet Gynecol 1984;5:672-4.
10. Monaghan JM. Bonney's gynaecological surgery. London-Ballière Tindall, 1986.
11. Lose G, Lindholm P. Imparied voiding efficiency and urinary retention after laparoscopic ventrosuspension ad modem Steptoe. Acta Obstet Gynecol Scand 1984;63: 371-2.

Pregnancy-induced hypertension and acute fatty liver of pregnancy: Atypical presentations

Mark A. Brown, MD, George Passaris, MB, BS, and Mark A. Carlton, MB, BS
Sydney, Australia

Severe pregnancy-induced hypertension and markedly elevated levels of serum alkaline phosphatase developed in a 29-year-old-woman in whom one pregnancy resulted in intrauterine death. Acute fatty liver of pregnancy developed with good fetal outcome in the next pregnancy. This case suggests that pregnancy-induced hypertension and acute fatty liver of pregnancy are part of a clinical spectrum. Moreover, classic abnormalities of liver function tests need not be present in acute fatty liver of pregnancy. (AM J OBSTET GYNECOL 1990;163:1154-6.)

Key words: Pregnancy-induced hypertension, acute fatty liver of pregnancy, alkaline phosphatase

Acute fatty liver of pregnancy and pregnancy-induced hypertension (preeclampsia) may comprise the clinical ends of a single disorder. In a recent review of nine patients with acute fatty liver of pregnancy concomitant preeclampsia was found in all cases[1] and a study of liver morphology found hepatocyte fat deposits of varying degrees both in women with preeclampsia and in those with acute fatty liver of pregnancy.[2]

We describe a patient who exhibited the continuum of this spectrum across two consecutive pregnancies and whose clinical diagnosis was made difficult by the single and progressive elevation of hepatic alkaline phosphatase levels without changes in other liver enzymes.

Case report

Hypertension (blood pressure 156/90) without proteinuria at 29 weeks' gestation developed in the third pregnancy of a 29-year-old woman who had had two previous miscarriages. Fundal height was consistent with only 24 weeks' gestation, and the patient had decreasing fetal movements. Uterine ultrasonographic examination confirmed intrauterine death of a fetus of approximately 21 weeks' gestation. Initial investigations showed the following values: hemoglobin, 12 gm/dl with some red blood cell fragments and tear drop poikilocytes; platelet count, 237,000; serum creatinine, 0.07 mmol/L; uric acid, 0.40 mmol/L; bilirubin, 3 μmol/L; alkaline phosphatase, 1040 U/L (normal < 120 U/L); aspartate transaminase, 28 U/L; γ-glutamyl transferase, 19 U/L; serum albumin, 38 gm/L. Alkaline phosphatase was not fractionated and was thought at that time to represent placental infarction.

The patient was delivered vaginally of a female weighing 800 gm. Placental pathology was normal with no evidence of placental infarction or retroplacental hemorrhage.

Further investigations demonstrated a normal activated partial thromboplastin time and serum was weakly positive for antinuclear antibodies (1:160) and was negative for anticardiolipin antibodies. Blood pressure rapidly returned to normal and the patient was reviewed 2 months later when repeat antinuclear antibodies, anticardiolipin antibodies, and double-stranded binding deoxyribonucleic acid (DNA) were negative. Serum complements, immune complexes, and intravenous pyelogram were normal and creatinine clearance was 98 ml/min with no proteinuria. Urinalysis and urine microscopy were normal. Liver function test results had returned to normal with the alkaline phosphatase level at 62 U/L.

The patient returned 6 months later, 10 weeks into her fourth pregnancy with the same partner. In view of her history of three unsuccessful pregnancies she was commenced on aspirin 100 mg daily from 14 weeks' gestation and the limited data available on this therapy were explained to her. Blood pressure was 116/68; both urinalysis and physical examination were normal. Antinuclear antibodies, double-stranded binding DNA, and anticardiolipin antibodies were negative. Liver function and renal function were normal.

The patient's blood pressure remained completely

From the Department of Renal Medicine and Obstetrics, St. George Hospital.
Received for publication March 22, 1990; revised April 21, 1990; accepted May 1, 1990.
Reprint requests: Dr. M. A. Brown, First Floor, Main Building, St. George Hospital, Kogarah, Sydney, NSW 2217, Australia.
6/1/22092

Table I. Biochemistry and hematology during and after pregnancy

Gestation (wk)	*BIL (μmol/L)*	*ALP (U/L)*	*AST (U/L)*	*GGT (U/L)*	*ALB (gm/L)*	*Hb (gm/dl)*	*Platelets (per $mm^3 \times 10^3$)*	*Creatinine (mmol/L)*	*UAC (mmol/L)*
30	5	890	16	6	37	12.1	217	0.06	0.25
32	8	1070	16	4	35	12.4	180	0.05	0.29
33	4	1120	10	2	35	12.2	186	0.05	0.26
34	7	1425	5	6	36	13.1	198	0.07	0.30
35	9	2210	9	5	33	12.7	211	0.07	0.30
Post partum (2 months)	16	72	34	8	48	14.7	217	0.08	0.31
Normal range (third trimester)	3-20	30-200	8-40	10-100	32-34	11-13	150-400	0.04-0.08	0.20-0.35

BIL, Bilirubin; *ALP*, alkaline phosphatase; *AST*, aspartate transaminase; *GGT*, γ-glutamyl transferase; *ALB*, serum albumin; *Hb*, hemoglobin; *UAC*, uric acid.

normal throughout pregnancy and at 30 weeks' gestation liver function tests were repeated in view of her past history. Her alkaline phosphatase level had risen without concomitant changes in bilirubin or other liver enzymes (Table I). Teardrop poikilocytes and macrocytosis were present on blood film with normal levels of serum B_{12} and folate. Uterine ultrasonographic examination confirmed adequate fetal growth and showed normal umbilical artery blood flow studies.

Over the ensuing 4 weeks plasma alkaline phosphatase levels rose steadily (Table I), despite cessation of aspirin. Antimitochondrial, antinuclear, and antismooth muscle antibodies were not detected, bile acid levels were normal and abdominal ultrasonographic examination showed normal liver architecture with no other abnormalities. Hepatitis B serum was negative for surface antigen and antibody, and cytomegalovirus antibodies were not detected. Electrophoresis and heat testing demonstrated the elevated level of alkaline phosphatase to consist almost totally of the hepatic isoenzyme.

An elective cesarean section was performed at 35 weeks' gestation and a healthy female weighing 2425 gm was delivered. The placenta and cord were normal histopathologically. Liver biopsy specimen obtained at cesarean section showed microvesicular fat (hematoxylin-eosin stain) in hepatocytes with some periportal sparing—features of acute fatty liver of pregnancy.

Two months postpartum the patient was completely well and liver function had returned to normal.

Comment

Whereas it has recently been suggested that pregnancy-induced hypertension and acute fatty liver of pregnancy may form a continuum of one disorder,[2] this is the first patient reported to our knowledge who demonstrates these two conditions in consecutive pregnancies. More important, the condition would not have been detected in her final pregnancy unless biochemical tests were performed, demonstrating an isolated rise in the level of alkaline phosphatase.

This patient's first pregnancy, complicated by hypertension, hyperuricemia, and abnormal liver function and resulting in intrauterine death, was consistent with a clinical diagnosis of severe pregnancy-induced hypertension. Although her next pregnancy progressed well clinically, the reemergence of elevated levels of alkaline phosphatase at 30 weeks' gestation was a worrying feature and was first presumed to reflect placental dysfunction. However, isoenzyme determination confirmed the alkaline phosphatase to be of hepatic origin despite no other abnormalities of liver function. The lack of hypertension, the normal platelet count, normal plasma urate, and lack of proteinuria meant that the increased alkaline phosphatase level was not a result of the development of pregnancy-induced hypertension in this pregnancy. To be sure that aspirin was not causing the elevated level of hepatic alkaline phosphatase, this medication was discontinued at 32 weeks' gestation but the alkaline phosphatase level continued to rise. Aspirin has been associated with the development of Reye's syndrome, with fatty vacuolization of the liver, but this disorder is generally confined to children younger than 15 years of age.

Inasmuch as elevation of alkaline phosphatase level was the only biochemical abnormality and levels of all other liver enzymes remained normal, the diagnosis of acute fatty liver of pregnancy was not made until the liver biopsy specimen was obtained at the time of cesarean section. Because the alkaline phosphatase level had been elevated in the previous pregnancy, it is possible that acute fatty liver co-existed with pregnancy-induced hypertension in that pregnancy, although the alkaline phosphatase level was not fractionated, a liver biopsy specimen was not obtained, and acute fatty liver

of pregnancy is said not to recur.[1] Therefore there was no clear-cut evidence of acute fatty liver of pregnancy in that pregnancy but there were some features that suggested that diagnosis in retrospect.

This case provides clinical support for the recent histologic proposal that pregnancy-induced hypertension and acute fatty liver of pregnancy form a continuum of one disorder.[2] More important, it demonstrates that elevations of serum bilirubin and aspartate transaminase levels need not occur for this condition to be present and the clinician should now be alert to the diagnosis of acute fatty liver of pregnancy even if the alkaline phosphatase level is the sole abnormality of liver function.

We wish to thank Dr. T. Cook, histopathologist, for his assistance with liver biopsy interpretation and Mrs. B. Smith for her assistance.

REFERENCES

1. Reily CA, Latham PS, Romera R. Duffy TP. Acute fatty liver of pregnancy. Ann Int Med 1987;106:703-6.
2. Minakami H, Oka N, Sato T, Tamada T, Yasuda Y, Hirota N. Preeclampsia: a microvesicular fat disease of the liver? Am J Obstet Gynecol 1988;159:1043-7.

Vibroacoustic stimulation and fetal behavioral state in normal term human pregnancy

Lawrence D. Devoe, MD, Cheri Murray, RN, David Faircloth, BS, and Edgard Ramos, MD
Augusta, Georgia

Vibroacoustic stimulation may affect human fetal behavior. Continuous graphic records of simultaneous ultrasonographic observations of fetal activities and electronic fetal heart rate tracings of 30 normal term fetuses were examined visually for the occurrence of behavioral state 30 minutes before and after 3 seconds of vibroacoustic stimulation. After vibroacoustic stimulation, the total time spent in state 1 decreased significantly, that spent in state 4 increased significantly, and times spent in state 2 and indeterminate state (no state established for at least 3 minutes) were unchanged. No fetus exhibited state 3 before or after vibroacoustic stimulation. State 4 occurred in 22 fetuses after vibroacoustic stimulation with a duration of at least 30 minutes in four fetuses, and was noted in all fetuses in pre–vibroacoustic stimulation state 1 and 11 of 16 fetuses in pre–vibroacoustic stimulation state 2. Fetal heart rate accelerations occurred within 10 seconds after vibroacoustic stimulation in 94% of the fetuses studied regardless of their prior behavioral state. The variation in the onset and duration of behavioral state responses in most fetuses after vibroacoustic stimulation may depend on previous behavioral state and could be important for interpretation of antenatal assessment that uses this stimulus. (Am J Obstet Gynecol 1990;163:1156-61.)

Key words: Vibroacoustic stimulation, fetal behavioral state, fetal heart rate, fetal movement

Vibroacoustic stimulation of the human fetus by means of an artificial electronic larynx has been advocated as an adjunct to antepartum fetal assessment.[1] Vibroacoustic stimulation has been used primarily to elicit fetal heart rate (FHR) accelerations and to reduce the time required for antepartum testing; it has been shown to have predictive values comparable to those of standard nonstress testing.[2] Although the mechanisms responsible for the FHR responses that follow vibroacoustic stimulation are uncertain, studies of individual fetal biophysical activities have shown that it is primarily associated with increased baseline FHR and frequency of FHR accelerations.[3,4] However, changes in other biophysical variables, such as fetal breathing movements and body movements, have been observed, suggesting that vibroacoustic stimulation may alter the organization of fetal behavioral state.[5,6] This hypothesis has also

From the Department of Obstetrics and Gynecology, Medical College of Georgia.
Received for publication November 28, 1989; revised May 2, 1990; accepted May 18, 1990.
Reprint requests: Lawrence D. Devoe, MD, Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA 30912.
6/1/22473

been supported by Lecanuet et al.[7] who applied sound stimuli of varying frequency, intensity, and repetition.

We undertook this study to evaluate the possible influence that vibroacoustic stimulation may exert on subsequent fetal behavior. We wished to determine whether normal term human fetuses capable of organized behavioral states would exhibit significant, predictable state changes after exposure to a standard vibroacoustic stimulus. We also sought to determine if a relationship existed between behavioral states that preceded and followed vibroacoustic stimulation.

Material and methods

Patient population. Thirty patients were recruited for this study from the general obstetric population at the Medical College of Georgia. All signed an informed consent and entered a study protocol approved by the Human Assurance Committee. Inclusion criteria for this investigation were as follows: (1) gestational age ≥37 and ≤42 completed weeks, (2) no evident maternal medical or obstetric complications, (3) singleton vertex presentation, without apparent anomalies and appropriate in size for gestational age, (4) intact amniotic membranes and adequate amniotic fluid volume as assessed by ultrasonographic visualization, and (5) no exposure to maternal drugs or medications other than prenatal vitamins.

Study protocol. All studies were conducted similarly between 8:30 AM and 11 PM in a quiet, darkened room. Each patient was observed approximately 2 hours after a 400 kcal breakfast, at complete rest for at least 1 hour beforehand, and positioned in a semi-Fowler position with a lateral hip displacement.

The study apparatus consisted of a standard linear array real time scanner, operating at 3.5 MHz (ADR model 4000/L, ATL Corporation, Tempe, Ariz.), an electronic FHR monitor, operating in the Doppler mode (Hewlett Packard model 8040A, Hewlett Packard Company, Andover, Mass.), an observer-operated event marker, and a specially programmed microcomputer (IBM XT, 640K RAM, IBM Corp., Boca Raton, Fla.) into which the event marker and FHR monitor were hard-wired. The software for acquiring and reconstructing the graphic display of fetal activities was developed in the Department of Biomedical Engineering at the Medical College of Georgia and has been previously described.[8] The artificial electronic larynx was a Corometrics vibroacoustic stimulator (Corometrics Corp., Wallingford, Conn.); its physical properties have been previously described[4] and it was adjusted to deliver a constant 3-second pulse. The operation of this device in free air, more than one meter removed from the maternal abdomen, was demonstrated for each patient in the hour before actual study.

Each observation session was conducted in the following manner. After the patient was admitted to the study room, no further eating or drinking was allowed. No smoking was permitted and no medications were administered. A 30-minute control period started during which simultaneous recording of FHR and entry of fetal body and breathing movements were performed. During periods when there were no observed fetal breathing or body movements, the scanning element was directed to the fetal head to verify the presence or absence of rapid eye movements. After 30 minutes of observation a 3-second vibroacoustic signal was delivered with the artificial larynx placed on the maternal abdomen, overlying the location of the fetal head. Immediately afterward a second 30-minute observation period was conducted and the same biophysical data were collected according to the same study techniques.

After the conclusion of each study the concurrent FHR and other biophysical data were recalled as a continuous graphic record and subjected to visual analysis by an independent observer. The 30-minute sequences were randomly varied for each patient so that the point at which vibroacoustic stimulation was performed was not known. The assignment of behavioral state followed a scheme, modeled after the criteria of Nijhuis et al.[9] as listed in Table I. State 1 corresponds to the "quiet sleep" state observed in the newborn; little body movement occurs, breathing patterns are regular, and the heart rate baseline shows little variability. State 2 is analogous to the rapid-eye-movement sleep state of the newborn; more body activity occurs, heart rate variability increases, and the breathing pattern becomes more irregular. State 3 may share features with the "quiet" wakeful state of the newborn; it is generally brief, rapid eye movements are present, but body movements are less frequent. State 4 is analogous to active wakefulness in the newborn; continuous body movements are present, breathing patterns are irregular, and the heart rate baseline shows marked variability with large amplitude oscillations. The assignment of behavioral state required that the defined state variables appeared together and persisted for a minimum of 3 minutes. When state criteria were not met for this length of time or were not determinable because of signal loss or failure of appropriate fetal visualization, the state was considered "indeterminate." State transitions were those epochs in which a fetus changed from one established state to another established state as characterized by the concurrent alteration of all observed state variables.

Grouped mean times spent in state and mean numbers of state transitions were calculated for the 30-minute periods before and after vibroacoustic stimulation. Only fetuses that established states during either 30-minute period were considered for final statistical

Table I. Criteria for fetal behavioral state*

State	*Fetal heart rate pattern*	*Body movements*	*Breathing movements*	*Eye movements*
1	Stable baseline; reduced variability; rare accelerations	Brief or absent	Infrequent, regular	Absent
2	Stable baseline; increased variability; accelerations episodic with movement	Episodic, gross trunkal flexion-extension	Frequent, irregular	Present
3	Wider oscillations than state 1; no accelerations	Absent	Infrequent, or absent	Present
4	Unstable, very wide oscillations; frequent episodes of tachycardia; large accelerations	Continuous, gross trunkal	Present, irregular	Present

*Adapted from Nijhuis et al.[9]

comparisons. Grouped mean parameters were compared with t tests with a p value < 0.05 considered significant. The outcome of each pregnancy was also examined for birth weight, gestational age at delivery, Apgar scores, umbilical arterial blood gas determinations, and the presence of neonatal complications.

Results

Study group. Twenty-eight of the 30 fetuses studied under this protocol had established behavioral states during the 30-minute control period or the poststimulation period and formed the basis for the statistical analysis presented in the remainder of this section. Mean (±SD) gestational age was 38.3 ± 1.2 weeks at study and 39.3 ± 1.2 weeks at delivery. Mean birth weight was 3430 ± 610 (SD) gm. No infant was below the 10th percentile in birth weight for gestational age, had 5-minute Apgar scores <7, had abnormal umbilical cord arterial blood gas values, or experienced any intrapartum or neonatal complications.

Behavioral state data. The mean duration of behavioral states of all fetuses immediately before and after vibroacoustic stimulation are shown in Fig. 1. No fetuses were observed in state 3 before or after vibroacoustic stimulation. After vibroacoustic stimulation the mean (±SD) time spent in state 1 decreased significantly from 9.7 ± 8.6 to 1.7 ± 3.5 minutes (p = 0.00004); the mean (±SD) time spent in state 2 did not change significantly (11.5 ± 10.1 vs 11.1 ± 8.4 minutes; p = 0.86); the mean (±SD) time spent in state 4 increased significantly (3.0 ± 5.4 to 11.4 ± 9.0 minutes; p = 0.00001). The mean (±SD) time spent in the indeterminate state did not change significantly (5.2 ± 5.8 vs 5.5 ± 4.8 minutes; p = 0.82). The mean (±SD) cumulative duration of time spent in any state established before vibroacoustic stimulation was 13.4 ± 9.7 minutes; the mean (±SD) persistence of all prestimulation states after vibroacoustic stimulation was 5.8 ± 6.4 minutes.

Twenty-two of the 28 fetuses had established behavioral states immediately before stimulation. Table II indicates the respective states maintained by fetuses within and after 5 minutes of vibroacoustic stimulation. During the 30 minutes after vibroacoustic stimulation, state changed in 16 of these 22 fetuses, while five of six fetuses previously in the indeterminate state established an identifiable state. Table II also shows features of behavioral state 4 after vibroacoustic stimulation. State 4 was eventually achieved by all four fetuses in state 1, 11 of the 16 fetuses in state 2, and five of the six fetuses in the indeterminate state. Both fetuses in state 4 before vibroacoustic stimulation remained in state 4. State 4 was achieved within 5 minutes by all fetuses previously in state 1, by seven of 11 fetuses previously in state 2, and by three of the five fetuses previously in the indeterminate state. The duration of post–vibroacoustic stimulation state 4 varied from 4 to 30 minutes for fetuses previously in states 1 and 2 and from 5 to 19 minutes for those previously in indeterminate states. However, mean durations of post–vibroacoustic stimulation state 4 were similar among fetuses previously in states 1 or 2. State 4 persisted in four fetuses for virtually the entire post–vibroacoustic stimulation period and was still present at the end of the 30-minute observation period for the three fetuses not in state 4 before vibroacoustic stimulation. State 4 was never achieved by five fetuses in pre–vibroacoustic stimulation state 2 and one fetus with pre–vibroacoustic stimulation indeterminate state.

The mean (±SD) numbers of state transitions before vibroacoustic stimulation (1.2 ± 0.94) and after vibroacoustic stimulation (1.5 ± 0.94) were not significantly different (p = 0.25). A maximum of three state transitions was observed either before or after vibroa-

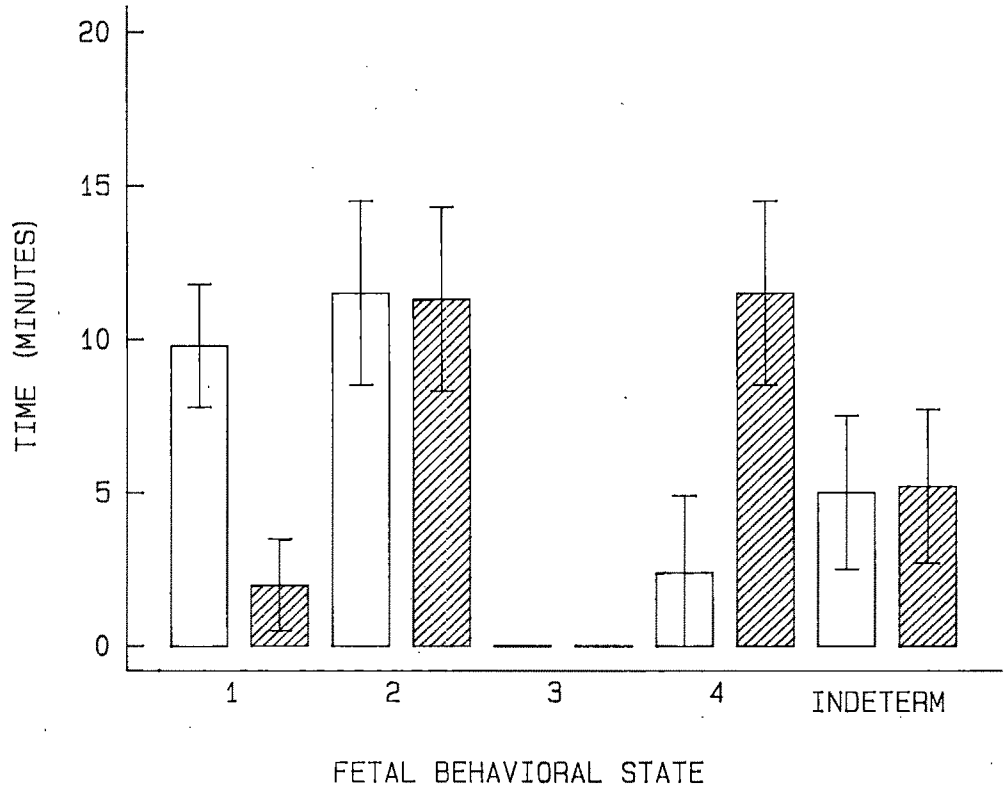


Fig. 1. Total times spent in various fetal states are shown for 28 fetuses. Prestimulation state is represented by *open bars* and poststimulation state is represented by *hatched bars. Indeterm,* Indeterminate state (no state established for at least 3 minutes). Standard errors are shown above each *bar.* No fetuses were observed in state 3 before or after vibroacoustic stimulation.

Table II. Behavioral state before and after vibroacoustic stimulation

	After stimulation				
Prestimulation state	*No. of fetuses in same state ≤5 min*	*No. of fetuses in same state >5 min*	*No. of fetuses achieving state 4*	*Onset of state 4* (min)*	*Duration of state 4* (min)*
1 (n = 4)	4	0	4	4.2 ± 2.1	13.0 ± 9.8
2 (n = 16)	16	5	11	6.6 ± 4.2	12.5 ± 7.8
4 (n = 2)	2	2	2	Immediate	21.5 ± 3.5
Ind. (n = 6)	6	1	5	10.6 ± 9.5	9.6 ± 5.6

Ind., Indeterminate. Duration refers to total time spent by all fetuses occupying that state after stimulation.
*Mean ± SD.

coustic stimulation. Within 10 seconds after vibroacoustic stimulation, 26 of 28 fetuses exhibited FHR accelerations in excess of 15 beats/min in amplitude and 15 seconds' duration; FHR accelerations were absent in one fetus in prestimulus state 2 and one fetus in the indeterminate state.

Comment

This study attempted to determine, under controlled environmental conditions and observation techniques, whether patterns of organized fetal behavior are changed after a brief vibroacoustic stimulus. Interest in the possible behavioral effects associated with vibroacoustic stimulation has been provoked by its application in the assessment of both antepartum[1] and intrapartum[10] fetal well-being, and the current uncertainties about its mechanism of action.

We observed apparent alterations in fetal behavioral state after the application of vibroacoustic stimulation, as shown by times spent in state and the nature of the

behavioral state achieved. In contrast with the previous studies of Gagnon et al.[3, 5] and our own group[4, 6] that examined individual behavioral variables, this study attempted to view integrated, concurrent changes in the variables that describe organized fetal behavior. Although rapid eye movements were not continuously tracked throughout each study, the previous data of Nijhius et al.[9] suggest that the coincidence of other state variables (e.g., FHR pattern, fetal body movements, and breathing patterns) are predictive of organized state in over 70% of observed epochs when gestational age is beyond 37 weeks, as was the case in all of our studies. Therefore as a qualification of our observations it is possible that coincidental cycles of fetal motility rather than "true" states might have been present in some instances when state 2 was assigned. Because of the characteristic concurrent patterns of fetal activity and FHR baseline associated with state 4, it is less likely that errors occurred in the identification of this state. Our data suggest that the fetal state established before vibroacoustic stimulation may influence the nature and duration of subsequent fetal states. Fetuses with a pre–vibroacoustic stimulation state 1 consistently and rapidly converted to a post–vibroacoustic stimulation state 4; fetuses with a pre–vibroacoustic stimulation state 4 maintained their behavior patterns. Most (57%) of our study fetuses were in state 2 before vibroacoustic stimulation, a finding consistent with that reported by Martin[11] for normal fetuses of this gestational age. The behavioral responses of fetuses in state 2 after vibroacoustic stimulation appear less consistent and one third of this group never established state 4 after vibroacoustic stimulation. These responses are consistent with those reported by Lecanuet et al.[7] despite the differences in the stimuli used in their study.

In view of our findings, the previous concept that fetal state may be "disorganized" by vibroacoustic stimulation[5] should probably be reconsidered and replaced by the concept that, under some circumstances, fetal behavioral state may be "reorganized" via evoked state transitions. The marked variability in poststimulus behavior for those fetuses not in states 1 or 4 suggest that the mechanism for FHR accelerative responses to this stimulus is not simply caused by state conversion. FHR accelerations may be observed in all states except state 3 and therefore their spontaneous occurrence does not require a change of state.[9] Because most study fetuses showed a brief tachycardiac response to vibroacoustic stimulation, regardless of the ultimate onset and duration of poststimulus state, the mechanism for this brief response may be independent of behavioral state or the function of higher cortical centers.[12] Alternately, it is possible that variations in vibroacoustic stimulation response may reflect variations in the intrauterine intensity of the stimulus, as demonstrated by Gerhardt et al.[13]

Our findings also have implications for clinical fetal assessment that may involve the adjunctive use of vibroacoustic stimulation. First, the apparent relationship between preexisting fetal state and subsequent vibroacoustic stimulation response suggests that the most immediate and dramatic poststimulus effects occur if the fetus has been previously in state 1. Second, most fetuses undergoing testing after 37 weeks are in state 2 before vibroacoustic stimulation and their subsequent behavioral responses are the least predictable. Third, a transient "response" in any individual behavioral variable, such as FHR baseline, after vibroacoustic stimulation should not imply that higher cortical centers of the fetus are intact. This does not invalidate the use of either the "fetal acoustic stimulation test"[1] or the more conventional nonstress test for assessing fetal condition. It simply suggests that the observation of FHR accelerations alone does not constitute a comprehensive statement of fetal condition. Finally, it is not clear that sustained episodes of any behavioral state, especially state 4, are of any consequence to fetal well-being; however, they may be important for the subsequent application and interpretation of other testing methods. It would seem prudent to infer from our experience that such sustained responses are not rare, may exceed 30 minutes, and should probably be considered if further antepartum biophysical testing immediately follows the use of vibroacoustic stimulation.

REFERENCES

1. Smith CV, Phelan JP, Paul RH, Broussard P. Fetal acoustic stimulation: a retrospective experience with the fetal acoustic stimulation test. Am J Obstet Gynecol 1985; 153:567-8.
2. Smith CV, Phelan JP, Platt LD, Broussard P, Paul RH. Fetal acoustic stimulation: II. A randomized clinical comparison with the nonstress test. Am J Obstet Gynecol 1986;155:131-4.
3. Gagnon R, Hunse C, Carmichael L, Fellows F, Patrick J. External vibratory acoustic stimulation near term: fetal heart rate and heart rate variability responses. Am J Obstet Gynecol 1987;156:323-7.
4. Devoe LD, Gardner P, Arnold P, Searle N. The effects of vibratory acoustic stimulation on baseline fetal heart rate in term pregnancy. Am J Obstet Gynecol 1989;160: 1086-91.
5. Gagnon R, Hunse C, Carmichael L, Fellows F, Patrick J. Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term. Am J Obstet Gynecol 1987;157:1375-81.
6. Devoe LD, Searle NA, Ruedrich DA, Castillo RA, Metheny WP. The effects of vibroacoustic stimulation on baseline heart rate, breathing activity, and body movements of normal term fetuses. Am J Obstet Gynecol 1989;161: 524-9.
7. Lecanuet J-P, Granier-Deferre C, Busnel M-C. Fetal cardiac and motor responses to octave-band noises as a function of central frequency, intensity and heart rate variability. Early Hum Dev 1988;18:81-93.
8. Devoe LD, Searle N, Searle J, et al. Computer-assisted assessment of the fetal biophysical profile. Am J Obstet Gynecol 1985;153:317-21.

9. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSCN. Are there behavioral states in human fetuses? Early Hum Dev 1982;6:177-95.
10. Smith CV, Nguyen HN, Phelan JP, Paul RH. Intrapartum assessment of fetal well being: a comparison of fetal acoustic stimulation with acid-base determinations. Am J Obstet Gynecol 1986;155:726-8.
11. Martin CB Jr. Behavioral states in the human fetus. J Reprod Med 1981;26:425-32.
12. Dawes GS. The central nervous control of fetal behavior. Eur J Obstet Gynecol Reprod Biol 1986;21:341-6.
13. Gerhardt KJ, Abrams RM, Kovaz BM, et al. Intrauterine noise levels in pregnant ewes produced by sound applied to the abdomen. Am J Obstet Gynecol 1988;159:228-32.

Successful conservative management of primary nonmetastatic cervical choriocarcinoma

Avraham Ben-Chetrit, MD,[a] Simcha Yagel, MD,[a] Ilana Ariel, MD,[b] David Zacut, MD,[a] Shlomo Shimonovitz, MD,[a] and Drorit Celnikier-Hochner, MD[a]

Jerusalem, Israel

Primary cervical choriocarcinoma is a rare disease; since 1915 only about 60 cases have been published. The case presented here can be defined as primary cervical choriocarcinoma since it fulfills all the criteria delineated previously. (Am J Obstet Gynecol 1990;163:1161-3.)

Key words: Cervical choriocarcinoma, hysterectomy, chemotherapy

Primary cervical choriocarcinoma is an extremely rare disease. This condition can be easily misdiagnosed as other, more common cervical pathologic conditions such as threatened abortion, benign or malignant lesions of the cervix, and even cervical pregnancy. Saito et al.[1] defined the criteria of ectopic choriocarcinoma as follows: (1) There is no primary focus of choriocarcinoma in the uterine corpus. (2) The histopathologic diagnosis should be confirmed as choriocarcinoma. (3) Extrauterine mole or normal intrauterine pregnancy should be excluded. (4) Intramural choriocarcinoma in the uterine corpus should not be considered as ectopic choriocarcinoma. According to the current literature the most common treatment is a combination of abdominal hysterectomy and chemotherapy. We present a case of primary cervical choriocarcinoma in a relatively young patient who desired to preserve her fertility; the disease was successfully treated by local excision of the lesion followed by chemotherapy.

From the Departments of Obstetrics and Gynecology[a] and Pathology, Hadassah Mount Scopus University Hospital.
Received for publication November 13, 1989; revised February 1, 1990; accepted April 26, 1990.
Reprint requests: Ben-Chetrit Avraham, MD, Department of Obstetrics and Gynecology, Hadassah University Hospital, Mount Scopus, 91240 Jerusalem, Israel.
6/1/21928

Case report

A 33-year-old, healthy, Jewish woman, married with three children, had three uneventful term pregnancies. Her last pregnancy was terminated 1 year before her present admission. She used an intrauterine contraceptive device and reported regular menses until 1 month before her admission when she noted irregular vaginal bleeding not related to coitus. On examination in another hospital the uterus was normal with no palpable adnexal masses. The intrauterine contraceptive device was removed. A 0.5 cm flat cervical lesion was found, and a biopsy was performed. Histologic examination was interpreted as a well to moderately differentiated invasive squamous cell carcinoma of the cervix. A month later the patient came to our hospital for second opinion and treatment. On admission, general physical examination gave normal findings. Pelvic examination showed a 0.5 × 1 cm cervical lesion. The uterus was normal with no palpable adnexal masses. Laboratory findings including peripheral blood count, platelet count, SMA-C test, erythrocyte sedimentation rate, creatinine clearance, and the markers carcinoembryonic antigen, α-fetoprotein, and CA 125 were within normal limits. The β-subunit human chorionic gonadotropin (β-hCG) level was 32 U (normal value, <10 U). Chest x-ray film was normal.

The patient underwent conization to determine the extent of the disease; the cone biopsy specimen measured 3 cm in diameter and had an irregular surface. The entire specimen was sectioned and examined mi-

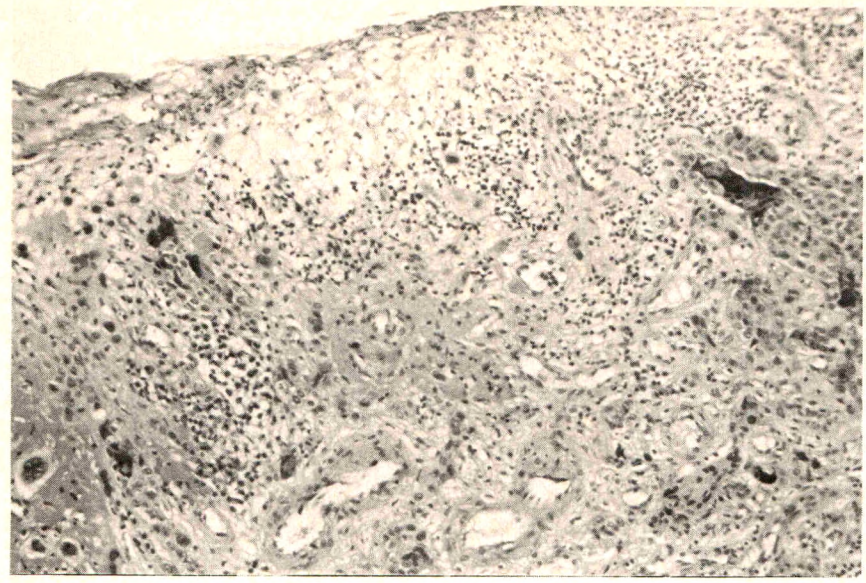


Fig. 1. Choriocarcinoma of uterine cervix. Overlying mucosa is ulcerated. (Hematoxylin and eoxin. ×110.)

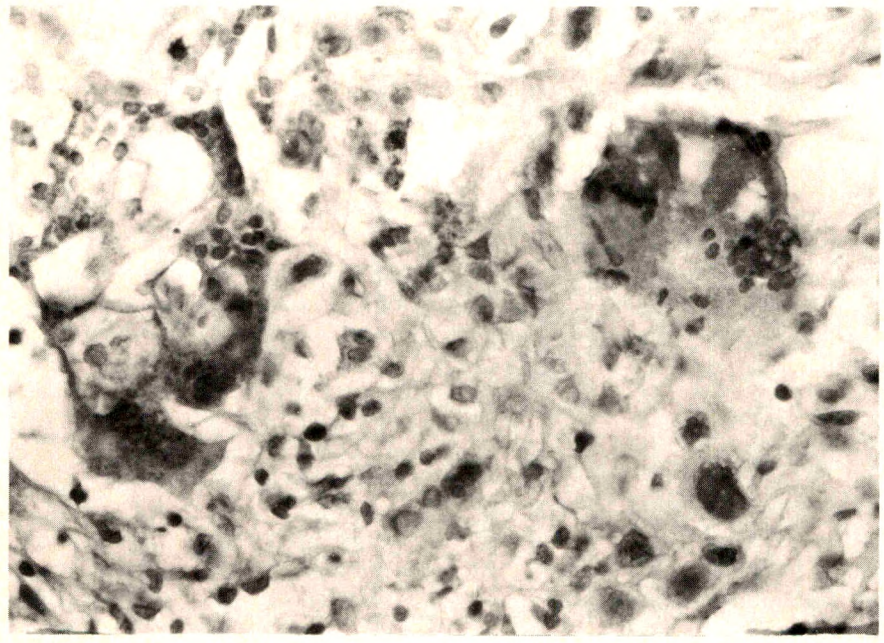


Fig. 2. β-hCG is demonstrated in syncytiotrophoblasts by immunoperoxidase technique. (×440.)

croscopically (Fig. 1). Tumor was found in the submucosa of the transformation zone between 12 and 3 o'clock. It consisted of masses of large mononuclear cells adjacent to groups of multinucleated cells and intermingled with blood lakes. A diagnosis of choriocarcinoma was made. The syncytiotrophoblasts stained positively for β-hCG by the immunoperoxidase technique (Fig. 2). Lines of resection were free of tumor. Reexamination of the previous biopsy specimen showed the same picture. Five days after the conization, serum β-hCG levels were <10 U; serum α-subunit human chorionic gonadotropin determination was not available in our hospital at that time. Further metastatic workup including brain and chest computed tomographic scan gave normal results. As the patient was interested in preserving her fertility, laparoscopy was performed and macroscopic lesions in the pelvis were excluded. Ten milliliters of clear fluid aspirated from the pouch of Douglas contained no malignant cells. Fractionated curettage showed a normal endocervix

and no evidence of trophoblastic disease. As conservative treatment has been adopted, the patient received two courses of intravenous methotrexate and folinic acid. Each course lasted 8 days and included four doses of 1 mg/kg methotrexate every other day and 0.1 mg/kg folinic acid on the alternating day, with a week's interval between the two courses. During and after the completion of the treatment the serum β-hCG levels, peripheral blood all count, and SMA-C test results were within normal limits. A repeated chest x-ray film did not show any evidence of metastasis.

A year after treatment, the patient's condition is good. Results of pelvic examination are normal, and β-hCG levels are <10 U/ml.

Comment

Our case was originally interpreted as a squamous cell carcinoma of the cervix. The latter occurs in the cervix with much greater frequency than choriocarcinoma. The cells of squamous carcinoma may have abundant eosinophilic cytoplasm as seen in cytotrophoblasts and may have more than one nucleus, thus resembling syncytiotrophoblast. It is not surprising therefore that a similar misdiagnosis has been described in another case by Tsukamoto et al.[2] The diagnosis of choriocarcinoma of the cervix should be confirmed by immunoperoxidase staining for β-hCG, as in our case.

Hysterectomy followed by chemotherapy is the treatment described in all the cases reported in the literature. In view that our patient wished to preserve her fertility and that the treatment for uterine choriocarcinoma during reproductive age may involve chemotherapy only, we decided to consider a conservative management. The negative metastatic workup supported our management.

In our opinion a nonmetastatic, primary cervical choriocarcinoma that occurs in a young patient who desires to preserve her fertility can be treated by local resection of the tumor followed by chemotherapy.

REFERENCES

1. Saito M, Azuma T, Nakamura K. On ectopic choriocarcinoma. World Obstet Gynecol 1965;17:459-84.
2. Tsukamoto N, Nakamura M, Kashimura M, Saito T. Primary cervical choriocarcinoma. Gynecol Oncol 1980;9:99-107.

The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemotherapy after surgical cytoreduction

Verda J. Hunter, MD, Lee Daly, PA-C, Michael Helms, PhD, John T. Soper, MD, Andrew Berchuck, MD, Daniel L. Clarke-Pearson, MD, and Robert C. Bast, Jr., MD
Durham, North Carolina

Fifty-four patients with advanced epithelial ovarian cancer were monitored with serial serum CA 125 levels after surgical cytoreduction and during multi-agent chemotherapy with cisplatin-containing regimens. CA 125 half-life of <20 days was associated with prolonged overall survival ($p < 0.015$). In those patients who eventually were found to be disease-free at surgical surveillance procedures, normalization of serum CA 125 levels to <35 U/ml within 65 days of primary operation also suggested an improved survival ($p < 0.059$). (AM J OBSTET GYNECOL 1990;163:1164-7.)

Key words: CA 125 half-life, ovarian cancer, prognosis

Serum CA 125 levels have been shown to correlate with response to therapy in patients with ovarian cancer.[1-3] Rapid and complete regression of tumor should be associated with a rapid decline in levels of the serum marker and might correlate with a favorable prognosis. In a report by van der Burg et al.,[4] patients with a CA 125 half-life of 20 days or more had a 3.2-fold higher progression rate and shorter median time to progression than did patients with a CA 125 half-life during the first month after chemotherapy correlated with progression-free survival. In an attempt to confirm and extend these potentially important observations, CA 125 half-life was calculated in patients with advanced stage epithelial ovarian cancer who had received primary chemotherapy after surgical cytoreduction.

Material and methods

Serial CA 125 levels were studied in 54 women with ovarian cancer who had undergone surgical cytoreduction followed by multi-agent chemotherapy at Duke University Medical Center between March 1984 and January 1989. Patients ranged in age from 25 to 86 years with a median of 57 years. Forty-four women had stage III disease and 10 had stage IV disease. All had epithelial tumors: 50 serous, one mucinous, and three undifferentiated carcinomas.

All patients underwent surgical cytoreduction at the time of initial diagnosis. Sixteen patients had tumor nodules <1 cm after surgery and 31 had nodules >1 cm. In seven patients who had received initial operation elsewhere, the precise status of residual disease was unknown. All patients received cisplatin-containing multi-agent chemotherapy at 3- to 4-week intervals according to Gynecologic Oncology Group protocols. Twenty-nine patients each received seven to nine cycles of cyclophosphamide (500 mg/m²) with cisplatin (50 mg/m²). Twenty patients received cyclophosphamide (500 mg/m²), cisplatin (50 mg/m²), and doxorubicin (50 mg/m²) for eight cycles. In six of these cases bacillus Calmette-Guérin was added to the regimen. Four patients received cyclophosphamide (1000 mg/m²) and cisplatin (100 mg/m²) during each of four cycles and one patient underwent six cycles of intraperitoneal cisplatin and intravenous cyclophosphamide chemotherapy.

Initial serum samples were obtained from 28 patients before cytoreductive operations, from 15 patients before the initiation of chemotherapy, and from 11 patients before the second course of chemotherapy. All patients were followed up with serial CA 125 value determinations and second samples were obtained in all cases within 90 days of operation. All sera were promptly separated and frozen at −70° C. Serum CA 125 was assayed according to directions in kits obtained from Centocor (Malvern, Pa.) as previously described.[1] All 54 patients initially had elevated CA 125 (>35 U/ml).

From the Departments of Obstetrics and Gynecology, Community and Family Medicine, Medicine, and Microbiology-Immunology and the Duke Comprehensive Cancer Center, Duke University Medical Center.
Funded in part by National Cancer Institute grant 5-R01-CA 39930.
Received for publication March 19, 1990; accepted May 18, 1990.
Reprint requests: Robert C. Bast, Jr., MD, Box 3843, Duke University Medical Center, Durham, NC 27710.
6/1/22432

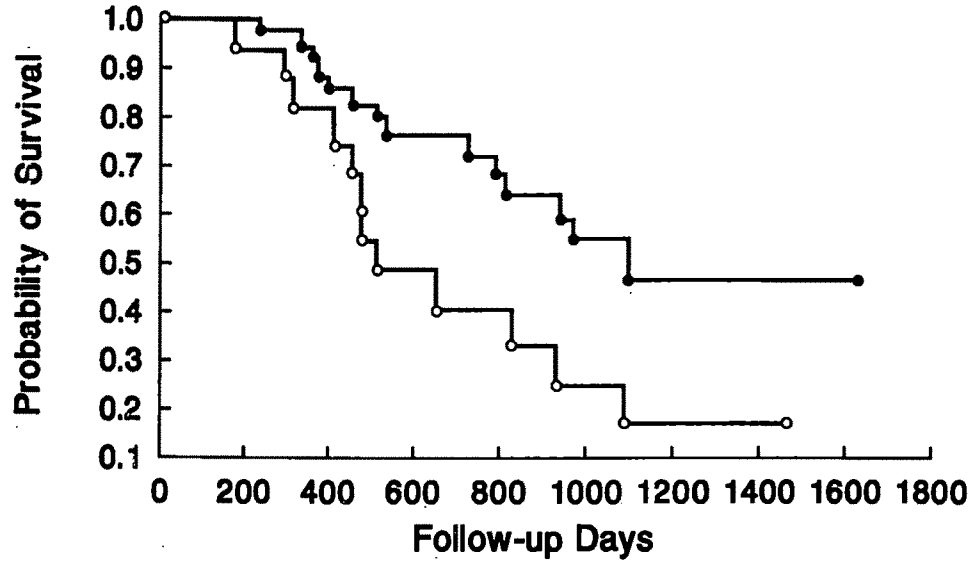


Fig. 1. Probability of survival for patients with ovarian cancer with serum CA 125 levels ≤20 U/ml *(solid circles)* and >20 U/ml *(open circles)*.

Days of follow-up were calculated from the date of operation to either the date of death or the date of last examination. CA 125 half-life for individual cases was calculated with the formula described by van der Burg et al.[4]:

$$T_{1/2} = \frac{dT}{\text{Log}_2\,(\text{CA 125-1}/\text{CA 125-2})}$$

where CA 125-1 is the prechemotherapy or preoperative value, CA 125-2 is the first normal CA 125 value or the lowest CA 125 value, if CA 125 did not normalize between 3 months after the start of therapy, and dT is the time between CA 125-1 and CA 125-2.

Patients were grouped as having CA 125 half-life values less than or more than different values. The interval to normalization of CA 125 (<35 U/ml) was also calculated for each patient. The date of progression was defined as the first date on which progressive, persistent, or recurrent tumor growth could be detected by clinical, radiologic, or surgical examination.[5] Second-look laparoscopies or laparotomies were performed in 39 patients. Four patients refused operation and 11 patients experienced progression before an operation could be performed. Median follow-up for all patients was 606 days and minimum follow-up was 305 days.

Survival curves were calculated and the resulting plots displayed through Kaplan-Meier analysis.[6] Tests of differences between the curves were made with the log-rank test. The test statistic consisted of a χ^2 with the degrees of freedom being one less than the number of curves being considered.

Results

Survival for 38 patients with serum CA 125 half-life values ≤20 days was compared with that for 16 patients with CA 125 half-life >20 days (Fig. 1). Overall survival was significantly greater in patients with a CA 125 half-life ≤20 days ($p < 0.015$, two-tailed). Median survival was approximately twofold greater in those individuals with the shorter CA 125 half-life. The correlation between half-life of serum CA 125 and time to disease progression did not achieve statistical significance ($p < 0.13$, two-tailed).

When all patients were considered, the interval required for CA 125 to fall below 35 U/ml did not correlate with disease-free or overall survival. However, if the 39 patients who underwent second-look surgical surveillance procedures were divided into subsets with and without residual ovarian cancer, an interesting trend was observed for those patients who were disease-free at second look (Fig. 2). In this group eight individuals whose serum CA 125 value normalized by 65 days after initial operation appeared to have improved survival when compared with five patients whose CA 125 level had not normalized. The number of patients in each group was, however, quite small and the differences only approached statistical significance ($p < 0.059$, two-tailed). The interval to normalization of CA 125 did not correlate with survival in those individuals with positive surgical surveillance procedures.

Comment

Lavin et al.[7] found that a failure to normalize CA 125 levels after 3 months of treatment was consistently as-

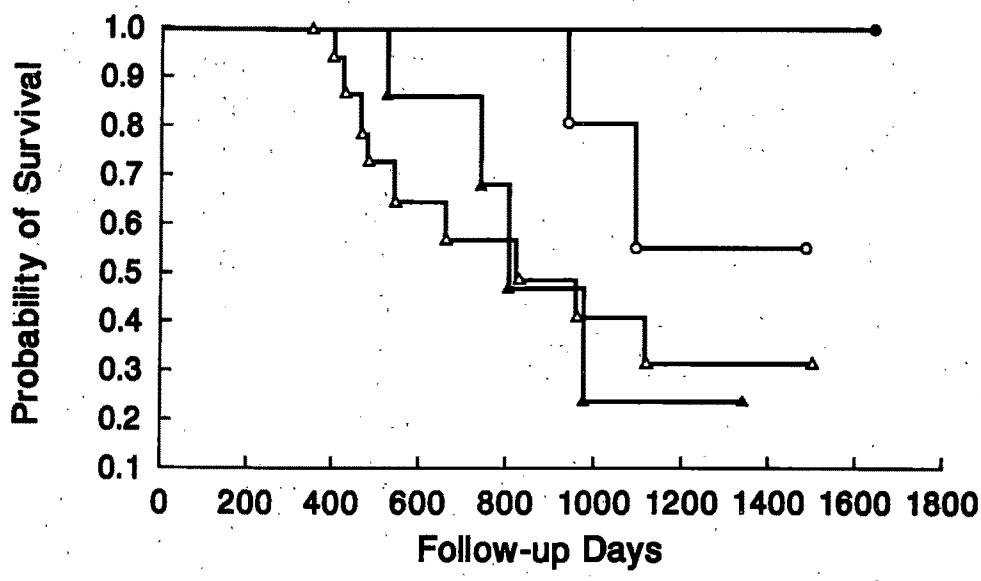


Fig. 2. Probability of survival for patients with ovarian cancer with positive *(triangles)* or negative *(circles)* surgical surveillance procedures. *Solid figures* indicate a fall in CA 125 level to >35 U/ml by 65 days.

sociated with persistence of ovarian cancer at completion of chemotherapy. All patients with negative surgical surveillance procedures had experienced a decline of CA 125 levels to <35 U/ml by 3 months. Consequently, the CA 125 level 3 months after treatment appeared to predict response to therapy in epithelial ovarian cancer. Van der Burg et al.[4] extended these observations, correlating the half-life of CA 125 with the rate of disease progression and the time to disease progression. In patients with a CA 125 half-life >20 days, a median time to disease progression of 11 months was found compared with 43 months for patients with half-life <20 days. The physiologic half-life of CA 125 has been estimated to be 4.8 days.[8] Larger values are thought to reflect the continued production of CA 125 by residual tumor. Rustin et al.[5] observed that disease-free survival was improved in those patients whose serum CA 125 had fallen sevenfold by 35 days after the initiation of chemotherapy.

This report extends these earlier studies to consider overall survival. Our experience in 54 patients with advanced ovarian cancer who had undergone surgical cytoreduction followed by multiagent cisplatin-based chemotherapy supports the observation that CA 125 half-life is a potentially important prognostic variable. Patients with a short CA 125 half-life had an improved overall survival when compared with patients with a CA 125 half-life >20 days.

However, a statistically significant correlation was not observed between CA 125 half-life and disease-free survival, in contrast to observations of van der Burg et al.[4] Our study population was still relatively small and statistical significance might have been achieved if a larger number of patients could have been studied. Our study was restricted to patients with stage III and IV disease, whereas the previous report[4] included patients with early stages of tumor spread. In early-stage disease surgical removal of tumor would provide complete cytoreduction and eliminate the major source of antigen before chemotherapy. In such patients a short half-life would be observed related to the operation rather than to the chemotherapy. Patients with early stage disease should also have the best prognosis and the longest time to tumor progression. The criteria used to determine disease progression constitute another difference between the two studies. Our use of positive surgical surveillance procedures to determine the date of disease progression may have minimized differences in disease-free interval between groups with rapid and slow CA 125 half-lives. In the earlier study by van der Burg et al.,[4] it was not stated how often surgical surveillance procedures had been performed or whether surgical findings had been used to determine the date of disease progression. Finally, patients may have been followed up at more frequent intervals after chemotherapy in the study by van der Burg et al., permitting a more precise determination of the date of clinical progression.

In our study a trend was observed toward correlation of survival with normalization of CA 125 by 65 days, but only in the subset of patients who had negative second-look surgical surveillance procedures. Lack of prognostic significance in the presence of persistent tumor may simply reflect the substantial importance of

macroscopic tumor at second look in determination of subsequent survival. A rapid fall in serum CA 125 levels need not preclude the presence of residual disease at second look. If tumors contained a small but significant fraction of drug-resistant cells, a majority of sensitive cells could be eliminated rapidly and still leave residual disease. Alternatively, rapidly proliferating cells might be quite sensitive to cytotoxic chemotherapy, but a small fraction of surviving cells regrow at a similarly rapid rate.

With cisplatin-based regimens a substantial fraction of patients will have disease recurrence within 2 years after a negative second-look procedure. However, the remainder will survive disease-free. In those patients with negative surgical surveillance procedures, the accurate identification of a subset who are destined to have disease recurrence would be of value in planning trials of more aggressive consolidation therapy. High-dose alkylator therapy with autologous bone marrow transplantation, for example, is likely to be most effective against minimal residual disease but is associated with significant morbidity and mortality.[9] A reliable correlate of poor prognosis in patients with a negative second-look operation would aid in selection of appropriate criteria for bone marrow transplantation or similarly aggressive consolidation chemotherapy.

Correlation of CA 125 half-life during primary therapy with subsequent overall survival is consistent with the importance of a response to the initial cytotoxic regimen and to a general lack of effective second-line therapy. Rate of response to primary chemotherapy has been correlated with the subsequent course of disease in leukemia, lymphoma, testicular carcinoma, and gestational trophoblastic neoplasia. On the basis of this report and from previous studies,[4, 5] this principle is likely to extend to epithelial ovarian carcinoma.

REFERENCES

1. Bast RC Jr, Klug TL, St. John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983;309:883-7.
2. Berek JS, Knapp RC, Malkasian GD, et al. CA 125 serum levels correlated with second-look operations among ovarian cancer patients. Obstet Gynecol 1986;67:685-9.
3. Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod 1989;4:1-12.
4. van der Burg ME, Lammes FB, van Putten WLJ, Stoter G. Ovarian cancer: the prognostic value of the serum half-life of CA 125 during induction chemotherapy. Gynecol Oncol 1988;30:307-12.
5. Rustin GS, Gennings JN, Nelstrop AE, Covarrubras H, Lambert HE, Bagshawe KD. Use of CA 125 to predict survival of patients with ovarian carcinoma. J Clin Oncol 1989;7:1667-71.
6. Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. J Am Stat Assoc 1958;53:457.
7. Lavin PT, Knapp RC, Malkasian G, Whitney CW, Berek JC, Bast RC Jr. CA 125 for monitoring of ovarian carcinoma during primary therapy. Obstet Gynecol 1987;69:223-7.
8. Canney PA, Moore M, Wilkensen PM, James RD. Ovarian cancer antigen CA 125: a prospective clinical assessment of its role as a tumor marker. Br J Cancer 1984;50:765-9.
9. Shpall EJ, Clarke-Pearson D, Soper JT, et al. High dose alkylating agent chemotherapy with autologous bone marrow support in patients with stage III/IV epithelial ovarian cancer. Gynecol Oncol [In press].

Management and outcomes of pregnancies complicated by human B19 parvovirus infection: A prospective study

John F. Rodis, MD, Dorothy L. Quinn, MS, G. William Gary, Jr., MPH, DrPH, Larry J. Anderson, MD, Sally Rosengren, MD, Matthew L. Cartter, MD, Winston A. Campbell, MD, and Anthony M. Vintzileos, MD

Farmington and Hartford, Connecticut, and Atlanta, Georgia

During a large statewide outbreak of fifth disease in Connecticut in 1988, 39 pregnant women were identified who had serologic evidence of recent human B19 parvovirus infection. The patients were followed up prospectively with targeted fetal ultrasonographic examinations to detect signs of fetal hydrops. Of these 39 pregnant women, 37 had healthy infants and two patients had miscarriages. None of the fetuses developed hydrops. We propose that pregnant women exposed to B19 parvovirus be tested for evidence of IgG and IgM B19-specific antibodies and that targeted fetal ultrasonography be considered when IgM antibodies are found. Percutaneous umbilical blood sampling and intrauterine transfusion can be considered in cases of B19 parvovirus-associated hydrops and anemia. The overall fetal loss rate in this prospective follow-up group was 5%. (AM J OBSTET GYNECOL 1990;163:1168-71.)

Key words: Parvovirus infection in pregnancy, targeted ultrasonography, hydrops

Intrauterine infection with human B19 parvovirus has been associated with fetal hydrops and death,[1-7] although the outcomes have been normal in the majority of cases. During a large and widely publicized statewide outbreak of erythema infectiosum (fifth disease), the childhood exanthem caused by B19 parvovirus, many pregnant women were exposed to the virus. More than 1000 pregnant women had blood tests for the presence of IgG and IgM parvovirus-specific antibodies. This article pertains to the pregnant women who were identified as having evidence of recent B19 parvovirus infection (i.e., B19 IgM-antibody–positive). The purpose of this report is to define the fetal risks associated with maternal B19 parvovirus infection during pregnancy in a prospective follow-up population and to propose a management protocol for pregnancies complicated by B19 parvovirus infection.

Material and methods

During a large statewide outbreak of erythema infectiosum from December 1987 through December 1988, many pregnant women contacted their physicians or the Connecticut Pregnancy Information Service at the University of Connecticut Health Center. Most of the women had either direct or indirect contact with a child with erythema infectiosum; many worked in schools with erythema infectiosum outbreaks. Patients were advised to have their blood tested for the presence of IgG and IgM parvovirus-specific antibodies. Testing was performed at the Centers for Disease Control by a previously described[8] enzyme-linked immunosorbent assay method.

Women with serologic evidence of recent infection by B19 parvovirus (i.e., B19 parvovirus IgM-antibody–positive) were advised to undergo targeted fetal ultrasonographic examinations, with special attention given to signs of fetal hydrops (polyhydramnios, placentomegaly, pericardial or pleural effusions, ascites, skin or scalp edema). Ultrasonographic examinations were performed at 1- to 2-week intervals until at least 6 to 8 weeks had elapsed from the date of maternal exposure, when it was known. In cases in which the timing of B19 parvovirus exposure was ill defined, ultrasonography was performed for 6 weeks from the time the B19 parvovirus IgM antibody–positive serum was drawn. This report includes only those patients whose pregnancies were uneventful at the time of serologic testing. In other words, to avoid selection bias, patients who had miscarriages who were then sent for B19 parvovirus testing are not included. Ultrasonography was performed by the Maternal-Fetal Medicine faculty of the University of Connecticut Health Center.

Pregnancy outcomes were obtained in all cases; in-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and the Division of Medical Genetics, Department of Pediatrics, University of Connecticut Health Center, Farmington; the Epidemiology Section, Connecticut Department of Health Services, Hartford; and the Division of Viral Diseases, Centers for Disease Control.

Received for publication March 6, 1990; revised May 3, 1990; accepted May 24, 1990.

Reprint requests: John F. Rodis, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06032.

6/1/22723

formation was obtained by the referring physicians or the pediatricians who had examined the newborns. Information obtained included birth weights, Apgar scores, gestational age at delivery, and documentation of major or minor congenital anomalies. In cases of spontaneous abortion fetal and placental tissues were examined histologically.

Results

During the study period 39 pregnant women were identified who had recent evidence of B19 parvovirus infection, all of whom were followed prospectively with fetal ultrasonographic examination. Pregnancy outcomes were available for all women. Thirty-seven (95%) were delivered of healthy newborns, 36 at term and one patient delivered of twins at 35 weeks. None of these fetuses had signs of fetal hydrops. One of the 38 infants had mild hypospadias. Two patients had spontaneous abortions for an overall pregnancy loss rate of 5%.

With regard to the two losses, one occurred in a patient with infertility who had suboptimal rises in quantitative β-human chorionic gonadotropin before her exposure to a child with erythema infectiosum early in the first trimester. She subsequently had falling β-human chorionic gonadotropin values and never required a dilation and curettage; thus no tissue was available for histologic examination. The other spontaneous abortion occurred in a woman exposed to B19 parvovirus at 10 weeks' gestation. Fetal heart tones were auscultated and serum was tested for B19 parvovirus antibodies. Two weeks later the patient had a spontaneous abortion. Histologic examination of the placenta revealed severe erythroblastosis. Because of formalin fixation of the products of conception, B19 parvovirus deoxyribunucleic acid probes could not be used to identify viral deoxyribonucleic acid.

Table I shows the gestational age at the time of serologic testing in the 39 women.

Table I. Distribution of gestational ages at time of serologic testing

Gestational age (wk)	*No.*	*% of total*
≤5	2	5.1
7-12	10	25.6
13-18	11	28.2
19-24	5	12.8
25-30	6	15.4
31-36	2	5.1
≥37	3	7.7
TOTAL	39	99.9

Comment

In most cases of maternal B19 parvovirus infection the fetus is not adversely affected, whereas in others—for reasons not well understood—miscarriages, fetal hydrops, and death may occur. The actual incidence of fetal death after maternal infection is not well established because previous studies have been retrospective and subject to selection bias. A retrospective review of all cases reported in the literature suggested a fetal loss rate of 38%,[4] whereas a recent report from West Germany found a 26% rate of hydrops in 39 pregnant women.[9] However, an unpublished prospective study from the United Kingdom suggests that the B19 parvovirus–associated fetal death rate after maternal infection is <10%.[10] To the best of our knowledge this study represents the first published report of a prospectively followed cohort of pregnant women with recent B19 parvovirus infection. Our overall 5% fetal loss rate is consistent with the findings of the unpublished British study.

If B19 parvovirus infection is considered from the larger public health prospective, three factors determine the risk of adverse pregnancy outcome. First is the overall susceptibility rate in the population. Previous studies have suggested that approximately 50% of adults in the United States have serologic evidence of past B19 parvovirus infection and are thus immune. During the period of our study 53% of pregnant women tested had evidence of past infection (M. L. C., unpublished data, 1988). The second factor to be considered is the actual rate of infection in these susceptible individuals in the setting of a community outbreak of erythema infectiosum. Previous epidemiologic studies would suggest that approximately 50% of susceptible household contacts become infected.[6] Data from one Connecticut community that was extensively studied by the Centers for Disease Control during the 1988 outbreak revealed that 19% of susceptible adult school personnel became infected in schools with erythema infectiosum outbreaks.[11] The third factor in the fetal risk equation is the actual B19 parvovirus–associated fetal loss rate in cases of maternal infection. Although two pregnancy losses occurred in 39 women (5%) in our study, one of these losses most likely was not related to B19 parvovirus infection, and therefore the B19 parvovirus–associated fetal loss rate may be as low as 2.5%

Approximately 50% of adults lack immunity to B19 parvovirus infection; as many as 20% of susceptible adults become infected after exposure in a school setting and 2.5% to 5% of these may have adverse pregnancy outcome once they become infected. Thus the overall B19 parvovirus–related fetal loss rate for pregnant women whose immunologic status is unknown was approximately 2.5 to 5/1000 pregnant women. As noted, we did not include patients who were tested after miscarriage in an effort to avoid selection bias. Thus there could have been other B19 parvovirus–related

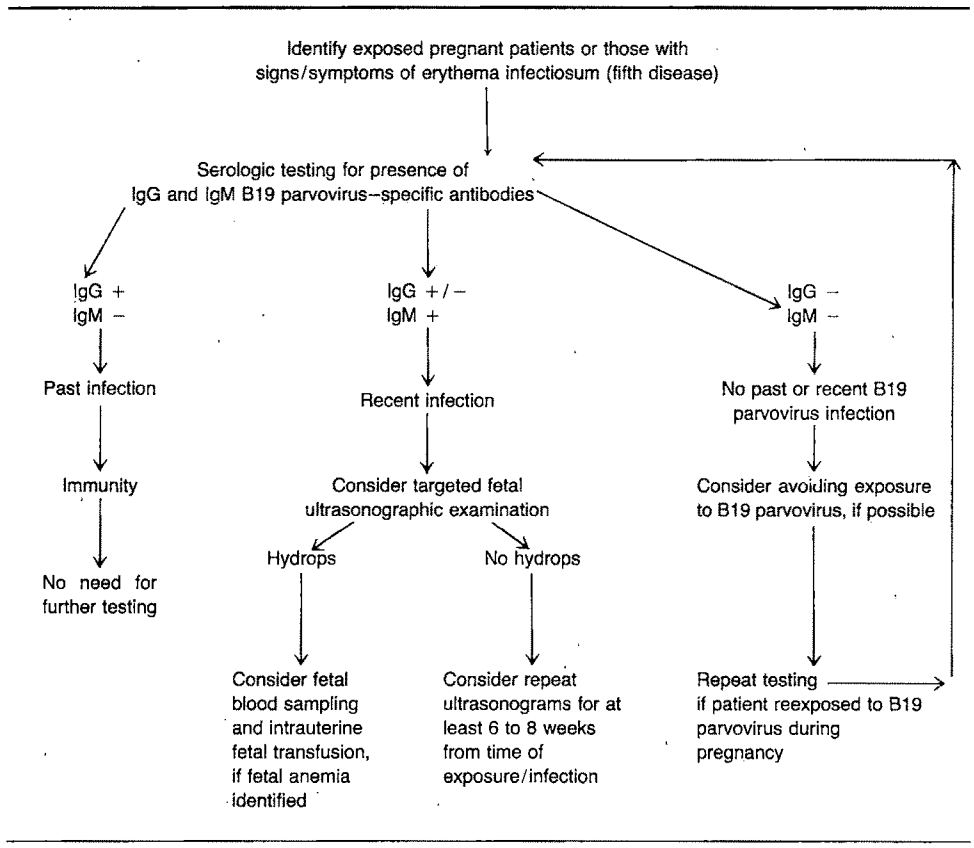


Fig. 1. Management protocol for B19 parvovirus exposure in pregnancy.

first-trimester losses. However, during the study period there were very few such cases. Therefore it is unlikely that their inclusion would have significantly altered the cited risks. Moreover, in keeping with the prospective nature of our study, we thought that these cases would best be excluded. Currently, serologic testing for B19 parvovirus infection is not widely available. The Centers for Disease Control and some state health departments offer B19 parvovirus antibody testing on a limited basis that includes testing pregnant women with exposure to B19 parvovirus or symptomatic B19 parvovirus infection.

We have previously suggested[4] that since the cause of fetal death in most cases appears to be profound fetal anemia, intrauterine transfusion might be a potential therapy in affected fetuses. Recently Schwarz et al.[9] reported intrauterine fetal blood transfusion in three affected fetuses with successful outcomes in all cases. This treatment has not been tested in a controlled study and thus is not recommended for routine treatment of B19 parvovirus–related fetal hydrops.[12]

We propose the management protocol depicted in Fig. 1. Pregnant women exposed to B19 parvovirus infection can be tested for B19 parvovirus antibodies. In general, maternal IgM antibodies appear 3 to 4 days after the onset of clinical illness, whereas IgG antibodies appear within 7 days. The IgM antibodies persist for 3 to 4 months and the IgG antibodies persist for years.[6-12] Therefore those who are positive for IgG antibodies and negative for IgM antibodies can be assured that they are at little, if any, risk from B19 parvovirus infection. Those with IgM antibodies have recently been infected and their fetus may be at risk of B19 parvovirus–related fetal death. The possibility of a B19 parvovirus adverse effect on the fetus can be monitored by directed ultrasonography and signs of hydrops including skin or scalp edema, ascites, hepatomegaly, placentamegaly, pleural or pericardial effusion, or altered fetal biophysical activities (e.g., decreased fetal movement) suggest that the fetus may be adversely affected by the infection. Percutaneous umbilical blood sampling can be considered to clarify the diagnosis by looking for severe anemia and evidence of B19 parvovirus infection. Fetal infection can be documented by demonstrating B19 parvovirus antigens or deoxyribonucleic acid and is strongly suggested by demonstrating eosinophilic inclusions and marginated chromatin characteristic of B19 parvovirus–infected erythroid precursors. Intrauterine blood transfusions can be considered to treat the affected fetus, although

its role in the treatment of this condition is yet to be defined and needs further study. Treatment with immune globulin is another therapeutic modality that merits study.

The fetus with a normal ultrasonogram on initial examination may develop abnormalities later; therefore consideration should be given to following the fetus with additional ultrasonograms. The timing of ultrasonically detectable hydrops in relationship to maternal infection and fetal death is not well understood; it is known, however, that fetal death usually occurs between 4 and 6 weeks but has occurred up to 12 weeks after symptomatic infection in the mother. The role of ultrasonography in follow-up of these pregnancies is not well defined and requires further study. Some authors[13, 14] have suggested that maternal serum α-fetoprotein levels be monitored because elevated values may identify the adversely affected fetus. One disadvantage of this approach is the many other causes of elevated maternal serum α-fetoprotein levels. Moreover, because the maternal serum α-fetoprotein test is primarily performed between 15 and 18 weeks' gestation, its value would be limited to cases of maternal infection during the late first or early second trimester.

Women susceptible to B19 parvovirus infection (IgG and IgM negative) should be advised of the risks of continued exposure to their fetus, and any decision to attempt to decrease this risk should be made by the women after consulting with other involved persons such as family members, employers, and public health officials.[12]

In conclusion, pregnant women with known B19 parvovirus exposure or signs or symptoms of fifth disease should be tested for presence of IgG and IgM parvovirus-specific antibodies. In patients with serologic evidence of recent infection (IgM positive), targeted ultrasonographic examinations and maternal serum α-fetoprotein testing may be helpful in identifying affected fetuses. It would appear that the fetal loss rate after maternal B19 parvovirus infection is ≤5%.

REFERENCES

1. Brown T, Anand A, Ritchie LD, Clewley JP, Reid TM. Intrauterine parvovirus infection associated with hydrops fetalis. Lancet 1984;1:1033-4.
2. Knott PD, Welply GAC, Anderson MJ. Serologically proved intrauterine infection with parvovirus. Br Med J 1984;289:1660.
3. Anand A, Gray E, Brown T, Clewley JP, Cohen BJ. Human parvovirus infection in pregnancy and hydrops fetalis. N Engl J Med 1987;316:183-6.
4. Rodis JF, Hovick TJ, Quinn DL, Rosengren SS, Tattersall P. Human parvovirus infection in pregnancy. Obstet Gynecol 1988;72:733-8.
5. Samra JS, Obhrai MS, Constantine G. Parvovirus infection in pregnancy. Obstet Gynecol 1989;73:832-4.
6. Anderson LJ, Hurwitz ES. Human parvovirus B19 and pregnancy. Clin Perinatol 1988;15:273-86.
7. Maeda H, Shimokawa H, Satoh S, Nakano H, Nunoue T. Nonimmunologic hydrops fetalis resulting from intrauterine human parvovirus B19 infection: report of two cases. Obstet Gynecol 1988;72:482-5.
8. Anderson LJ, Tsou C, Parker RA, et al. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. J Clin Microbiol 1986;24:522-6.
9. Schwarz TF, Roggendorf M, Hottentrager B, et al. Human parvovirus B19 infection in pregnancy. Lancet 1988; 1:566-7.
10. Public Health Laboratory Service Working Party on Fifth Disease. Study of human parvovirus (B19) infection in pregnancy. Comm Dis Rep (UK) 1987;20:3.
11. Gillespie SM, Cartter ML, Asch S, et al. Human parvovirus B19: occupational risk for school and day-care personnel during an outbreak of erythema infectiosum. JAMA 1990;263:2061-5.
12. Centers for Disease Control. Risks associated with human parvovirus B19 infection. MMWR 1989;38:81-97.
13. Carrington D, Gilmore DH, Whittle MJ, et al. Maternal serum α-fetoprotein: a marker of fetal aplastic crisis during intrauterine human parvovirus infection. Lancet 1987;1:433-5.
14. Bernstein IM, Capeless EL. Elevated maternal serum α-fetoprotein and hydrops fetalis in associated with fetal parvovirus B19 infection. Obstet Gynecol 1989;74:456-7.

Colon cancer in pregnancy with elevated maternal serum α-fetoprotein level at presentation

Whitney Gonsoulin, MD, Brian Mason, MD, and Robert J. Carpenter, Jr., MD
Houston, Texas

A case of colon cancer in pregnancy is presented in which the maternal serum α-fetoprotein level was elevated. Failure to evaluate colon cancer as a cause of the elevated maternal serum α-fetoprotein may have accounted for the poor outcome. (Am J Obstet Gynecol 1990;163:1172-3.)

Key words: Colon cancer, pregnancy, α-fetoprotein

The use of maternal serum α-fetoprotein (AFP) testing as a screening method for the early detection of fetal anomalies and chromosomal defects has increased in recent years. However, a variety of nonobstetric diseases may also elevate maternal serum AFP values and this knowledge can assist the practitioner in the early detection of these disorders. We present a case report of a patient with adenocarcinoma of the colon during pregnancy in which the first abnormality noted was elevated maternal serum AFP.

Case report

A 33-year-old white primigravid woman had routine prenatal care without apparent complications. At 16 weeks the maternal serum AFP level was 198.6 mg (6.2 multiples of the median). Ultrasonography was normal at 17 weeks and amniocentesis showed normal level of amniotic AFP and negative acetylcholinesterase. Chromosome analysis showed a normal 46,XY pattern.

At 23 weeks' gestation the patient was seen for complaints of epigastic pain. She denied nausea or vomiting, change in bowel habits, or melena. Initial medical therapy was unsuccessful. Physical examination showed a mass in the epigastrum and left upper quadrant. A liver ultrasound demonstrated a large liver mass. Magnetic resonance imaging showed an enlarged liver with the appearance of a multifocal hepatoma occupying both right and left lobes. A liver biopsy was performed at 25 weeks that revealed an adenocarcinoma of unknown origin. Carcinoembryonic antigen was significantly elevated at 630 ng/dl and gastrointestinal malignancy metastatic to the liver was considered.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Baylor College of Medicine.
Received for publication March 6, 1990; revised March 27, 1990; accepted May 1, 1990.
Reprint requests: R. J. Carpenter, Jr., MD, 6550 Fannin St., 901, Houston, TX 77030.
6/1/22090

At 25 weeks' gestation, the patient was transferred to our tertiary center with complaints of abdominal pain, shortness of breath, and vomiting. Physical examination was remarkable for a grossly distended abdomen with ascites. Colonoscopy revealed a large fungating, friable mass approximately 15 cm in length in the midtransverse colon occluding 80% of the lumen. Biopsies confirmed invasive adenocarcinoma similar to that of the liver biopsy. Obstetric ultrasonography was performed and confirmed a viable singleton pregnancy without anomalies.

The patient's physical condition deteriorated rapidly and she became progressively more lethargic and anorexic. On the seventh hospital day, fetal heart tones could not be auscultated and fetal death was confirmed by ultrasonography. Labor was induced without difficulty. Postpartum the patient became oliguric and developed gram-negative septicemia confirmed by blood cultures. Despite antibiotic therapy and replacement of clotting factors, the patient's coagulopathy persisted and progressive oliguria and ascites developed. The patient died after cardiopulmonary arrest on hospital day 21.

Necropsy on the fetus revealed an 890 gm male infant without congenital abnormalities. Study of the placenta showed acute inflammation of the decidua and chorion without metastases. Autopsy of the mother revealed a 6530 gm liver with 95% of the liver parenchyma replaced by necrotic tumor and an adenocarcinoma of the transverse colon measuring 4.5 × 6 cm.

Comment

As more women are screened with maternal serum AFP level determinations, physicians are confronted with unexplained elevations of maternal serum AFP values. Causes for elevation include a variety of obstetric conditions including neural tube defects, abdominal wall defects, multiple gestation, Rh isoimmunization, improper dates, and intrauterine death. In addition, there are a number of nonobstretic disease processes that can cause elevations in maternal serum

AFP such as hepatitis, infectious mononucleosis, liver, stomach, and colon cancer.[1]

In this case, marked elevations in the maternal serum AFP level were probably related to the primary adenocarcinoma or the hepatic metastases. Unfortunately, symptoms of early pregnancy can be confused with the symptoms of colon cancer, which include rectal bleeding, constipation, persistent abdominal pain, or a rectal or an abdominal mass. Screening tests such as carcinoembryonic antigen assay are often elevated in pregnancy and are therefore of limited use in diagnosis of colon cancer in pregnancy. The majority of colon cancers occur below the peritoneal reflection and can be diagnosed by rectal examination. For higher lesions, sigmoidoscopy or colonoscopy may be necessary. Early detection and treatment greatly improves the prognosis of this disease.

The incidence of colon cancer in pregnancy is estimated to be in 1/50,000 to 1/100,000 pregnancies[2]; however, this number may be expected to rise as an increasing number of older women become pregnant. Unexplained elevations in maternal serum AFP, particularly in the face of normal amniotic fluid AFP, necessitates further workup to rule out potentially treatable causes.

REFERENCES

1. Lau H, Linkins S. Alpha-fetoprotein. AM J OBSTET GYNECOL 1976;124:533.
2. Allen H, Nisker J. Cancer in pregnancy—therapeutic guidelines. Mount Kisco, New York: Futura Publishing, 1986:281.

Effects of hypertension on pregnancy monitoring and results

Jack W. Lenox, MD, Valentine Uguru, MD, and Luis A. Cibils, MD
Chicago, Illinois

The effect of severity of hypertension on fetal heart rate tracing changes and neonatal outcomes was evaluated on all patients with hypertension seen in 1980 and 1981 (666 cases, 10% of the pregnant population) in the Chicago-Lying In Hospital. The patients were grouped according to severity of hypertension, and the fetal heart rate monitoring, drugs administered, mode of delivery, and neonatal outcome were analyzed. Half of the patients (326) had mild hypertension and 13% (87) had severe hypertension; the remainder (253) had moderate hypertension. There were 49% primiparous and 51% multiparous women. The diagnosis of preeclampsia was made in 76% of cases, and chronic hypertension in 19%. Only 2% of the total were premature by dates, but 47% of this group were among the severe group. Oxytocin was given to 50%, whereas delivery was spontaneous in 56% of cases, and by cesarean section in 22%. This was higher among the severe hypertension group (37%), and the prematurity rate was 47%. Nonstress testing was done in one third of cases and only nonreactivity was associated with neonatal death. Neonatal depression (Apgar score < 6 at 5 minutes) was significantly associated with intrapartum fixed baseline and late decelerations; these were the best predictors of fetal outcome. The administration of magnesium sulfate, hydralazine, meperidine, or morphine did not predictably affect the fetal heart rate pattern. The perinatal mortality was 21‰ in the mild group and 36‰ and 138‰, respectively, among moderate and severe cases of hypertension. Close antepartum and intrapartum surveillance, including proper fetal monitoring, should help to reduce risks for mother and fetus through timely intervention. (AM J OBSTET GYNECOL 1990;163:1173-9.)

Key words: Hypertension, fetal heart rate, monitoring

Pregnancies complicated by hypertension have been extensively studied, with increased complications noted in cases of intrauterine growth retardation,[1-3] fetal distress,[4-7] and perinatal mortality.[2,3,8,9] Prospective studies have shown that the likelihood of poor fetal outcome increases as the severity of hypertension increases.[8,10] The most common reason suggested for these complications postulates a decreased perfusion of the intervillous space as a result of peripheral vasoconstriction

From The Chicago-Lying In Hospital, The University of Chicago.
Received for publication February 27, 1990; accepted May 18, 1990.
Reprint requests: Luis A. Cibils, MD, 5841 S. Maryland Ave., Chicago, IL 60637.
6/1/22480

Table I. Distribution by gestational age

Severity of hypertension	*No.*	*≤28 wk*	*29-31 wk*	*32-36 wk*	*37-41 wk*	*>42 wk*	*% Prematurity*
Group 1	326 (49%)	2 (14%)	3 (14%)	11 (24%)	289 (52%)	21 (62%)	5
Group 2	253 (38%)	4 (29%)	7 (31%)	13 (29%)	218 (40%)	11 (32%)	9
Group 3	87 (13%)	8 (57%)	12 (55%)	21 (47%)	44 (8%)	2 (6%)	47
TOTAL	666 (100%)	14 (2%)	22 (3%)	45 (7%)	551 (83%)	34 (5%)	

that ultimately produces placental insufficiency. This decreased perfusion would be reflected during the antenatal period or in labor by abnormal fetal heart rate (FHR) patterns that are known to occur in some hypertensive pregnancies.[4] This study is a retrospective analysis of the effect of the severity of hypertension on FHR patterns and fetal outcome, as well as an evaluation of commonly administered medications and their effects on intrapartum FHR patterns. The minimal blood pressure reading chosen to indicate hypertension of pregnancy was 125/75 mm Hg in view of the relatively recently acquired knowledge that in the last two trimesters of human pregnancy the normal blood pressure fluctuates around 100/60 mm Hg with very narrow ranges, and that preeclampsia has been regularly described with readings of 125/75 mm Hg.[11]

Material and methods

The study group consisted of all pregnant women with hypertension, defined as blood pressure of >125/75 mm Hg, after the fifth month of gestation. All patients were delivered at the Chicago-Lying In Hospital between Jan. 1, 1980, and Dec. 31, 1981. The medical records of the study group were reviewed (by U. V.) of the 666 patients who fulfilled the criteria, and 80 parameters were extracted. This population was divided into three subgroups based on the severity of hypertension. Patients in group 1 had blood pressure recordings between 125/75 and 135/85 mm Hg (n = 326, 49% of cases); group 2 patients had blood pressure readings from 136/86 up to 150/100 mm Hg (n = 253, 38%); group 3 patients had blood pressure measurements >150/100 (n = 87, 13%).

The intrapartum FHR tracings were reviewed (by J. W. L.) in 451 of these patients for whom they were available. These FHR tracings were analyzed to determine the type of decelerations (if any) present (early, variable, late), the baseline rate (normal, 120 to 150 beats/min), and the alterations of the baseline pattern (normal oscillations, fixed, saltatory).[4]

Prenatal care was given at Chicago-Lying In Hospital to 564 patients (85%); of the remainder, 61 patients (9%) received either no prenatal care or prenatal care was provided at local clinics or offices, and 41 (5%) were transferred from other hospitals. Maternal ages ranged between 13 and 45 years; 29% (n = 191) were between 13 and 19 years; 47% (n = 310) between 20 and 29 years; 22% (n = 149) between 30 and 39 years; and 2% (n = 16) were over 40 years of age. There were approximately equal numbers of primiparous (n = 327) and multiparous (n = 339) patients. The majority of patients were black (n = 633), with whites (n = 25) and other ethnic backgrounds (n = 8) present in much smaller numbers.

The patients were given a diagnosis of preeclampsia (n = 503), eclampsia (n = 4), chronic hypertension (n = 128), renal disease (n = 19), and "other" diagnosis (n = 12), including sickle cell disease, epilepsy, idiopathic thrombocytopenic purpura, etc., according to standard clinical criteria. Labor was spontaneous in 241 patients (36%), induced in 200 (30%), and enhanced in 130 patients (20%). There was no labor in 75 cases (11%), and 20 cases were uncoded (3%). Preterm deliveries (≤36 weeks) occurred in 81 patients (12%), whereas 551 pregnancies (83%) were term (37 to 41 weeks), and 34 (5%) were ≥42 weeks from the last menstrual period.

All data from the chart review and the data from the FHR tracings analysis were entered into a computer for statistical analysis, usually with the χ^2 test.

Results

The total number of women delivered at Chicago-Lying In Hospital during the study period (1980 and 1981) was 6648, of which 666 were classified as hypertensive, for a 10% incidence of hypertension (as defined here) in the population.

Table I shows the gestational ages of the patients in each study group. Patients in groups 2 and 3 accounted for 80% of all preterm deliveries (≤36 weeks) and 85% of deliveries at ≤32 weeks' gestational age. Table II shows the type of delivery for each group; it is of interest that, as the severity of hypertension increased there was an increase in the cesarean section rate, a decrease in the spontaneous delivery rate and, essentially, no difference in the rate of forceps or vacuum deliveries ($p < 0.001$). Furthermore, 22% of the cesarean sections occurred in group 3 patients (Table III), 37% of whom were delivered by this route (Table II), even though this group constitutes only 13% of the total population. The indications for intervention were analyzed for each study group among the patients who

Table II. Type of delivery

Groups	*Spontaneous*	*Forceps*	*Vacuum*	*Cesarean section*
1 326 (49%)	189 (58%)	75 (23%)	8 (2%)	54 (17%)
2 253 (38%)	145 (57%)	43 (17%)	7 (3%)	58 (23%)
3 87 (13%)	35 (40%)	18 (21%)	2 (2%)	32 (37%)
TOTAL 666	369 (56%)	136 (20%)	17 (2%)	144 (22%)

df 6; $p < 0.001$.

Table III. Indications for cesarean sections

Group	*No.*	*Maternal*	*Fetal*	*Failure to progress*	*Combination*
1	54 (37%)	26 (48%)	12 (22%)	8 (15%)	8 (15%)
2	58 (40%)	19 (33%)	16 (28%)	13 (23%)	9 (16%)
3	32 (22%)	3 (9%)	15 (45%)	8 (24%)	7 (21%)
TOTAL	144 (100%)	48 (33%)	43 (30%)	29 (20%)	24 (17%)

df 6; $p < 0.01$.

had cesarean section (Table III). A higher percentage of cesarean sections was performed in patients in group 3 for fetal indications than in groups 1 or 2. The majority of these cases involved fetal distress, determined by either antepartum heart rate monitoring (nonstress test–contraction stress test), falling estriol levels, intrapartum decelerations, or low fetal scalp pH values. The majority of patients in groups 1 and 2 who had cesarean deliveries for maternal indications were patients with previous cesarean deliveries.

A higher percentage of group 3 fetuses exhibited late decelerations (11%) as compared with those in group 1 (5%) or 2 (4%), although this difference was not significant. A similar occurrence was observed at evaluation of the baseline rate, with no significant differences among the hypertensive groups, although a higher percentage of group 3 fetuses (13%) did have baseline tachycardia than those in groups 1 (7%) and 2 (8%). As the severity of hypertension increased, significantly more fetuses within each group exhibited a sustained fixed baseline ($p < 0.03$) as shown on the left side of Fig. 1.

To determine whether the duration of hypertension influenced the FHR patterns recorded during labor, a comparison was made between patients with preeclampsia (presumably shorter time) and those diagnosed as having chronic hypertension. There were no differences between these two groups in the incidence of type of decelerations observed, the baseline rate, or baseline alterations. The data shown in Fig. 1 suggest that the degree of hypertension is a more important determinant of abnormal FHR patterns than is the duration of hypertension.

The effects of intrapartum medications on the FHR patterns were analyzed for the various FHR parameters and were grouped according to the medications the patients received during labor. There were no significant differences in the types of decelerations recorded when patients who received no medication were compared with those who received either magnesium sulfate, hydralazine, or sedatives, although a higher percentage of fetuses in the hydralazine group did have late decelerations compared with the other groups. Similarly, there were no differences when the baseline rate was analyzed among the patients who received no medications and those who were medicated. However, when analyzed for effect on baseline patterns a significantly higher ($p < 0.001$) number of fixed baseline tracings were found in the groups that received hydralazine and magnesium sulfate than in the groups that received no medication or sedatives (Fig. 2). Because an increase in fixed baseline tracings was also observed as the severity of hypertension increased (Fig. 1), it may have been possible that administration of magnesium sulfate and hydralazine accounted for this increase. However, when each hypertensive group was analyzed for the type of intrapartum medications received (Table IV), the percentage of patients who received magnesium sulfate was nearly the same in each group, whereas the percentage of patients who received hydralazine did increase with increasing hypertension. The last finding suggests that although magnesium sulfate does not appear to affect baseline, the conditions that make it necessary to administer hydralazine may influence baseline patterns.

The results of nonstress tests performed on each hypertensive group are listed in Table V. There were significantly more nonreactive tracings in patients in group 3 than in patients in group 1 or 2 ($p < 0.005$); in fact, 31% of all patients in group 3 were nonreactive, and there were two neonatal deaths among this group. In group 2 patients there was one neonatal death

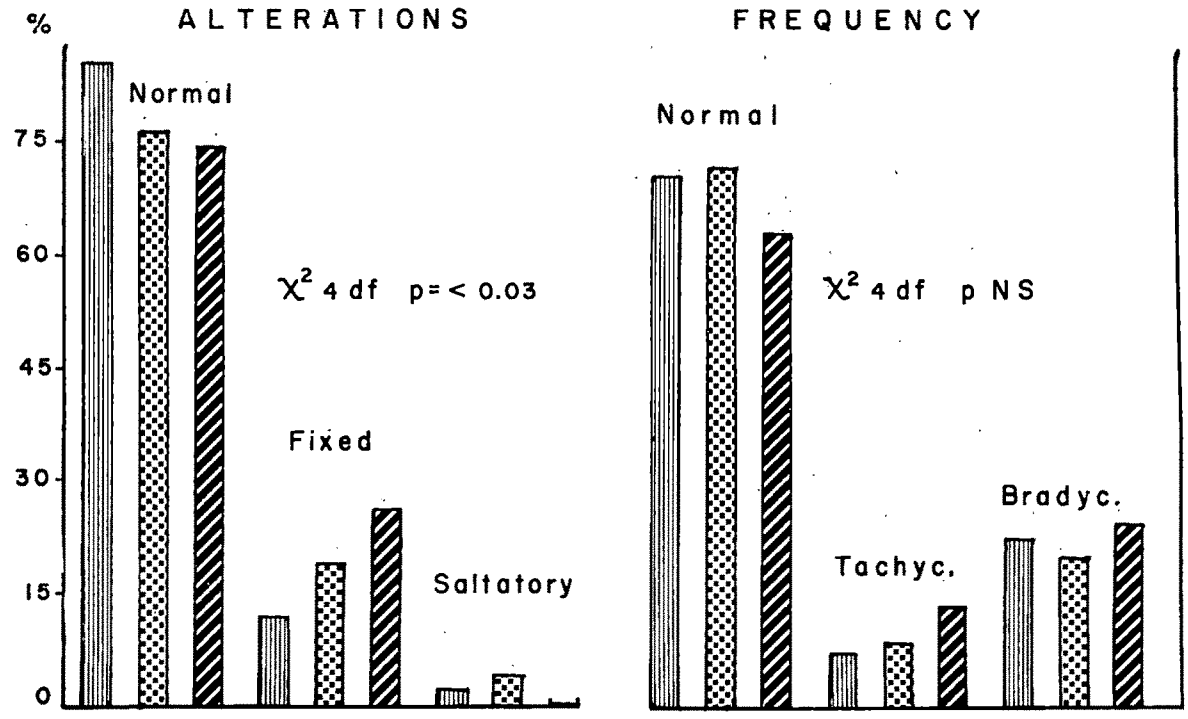


Fig. 1. Incidence of FHR baseline alterations and frequency by severity of hypertension is shown in bars. *Thin vertical stripes,* group 1; *dotted bars,* group 2; *thick oblique stripes,* group 3. On left side alterations occur in significantly different proportions. On right baseline frequency is not different.

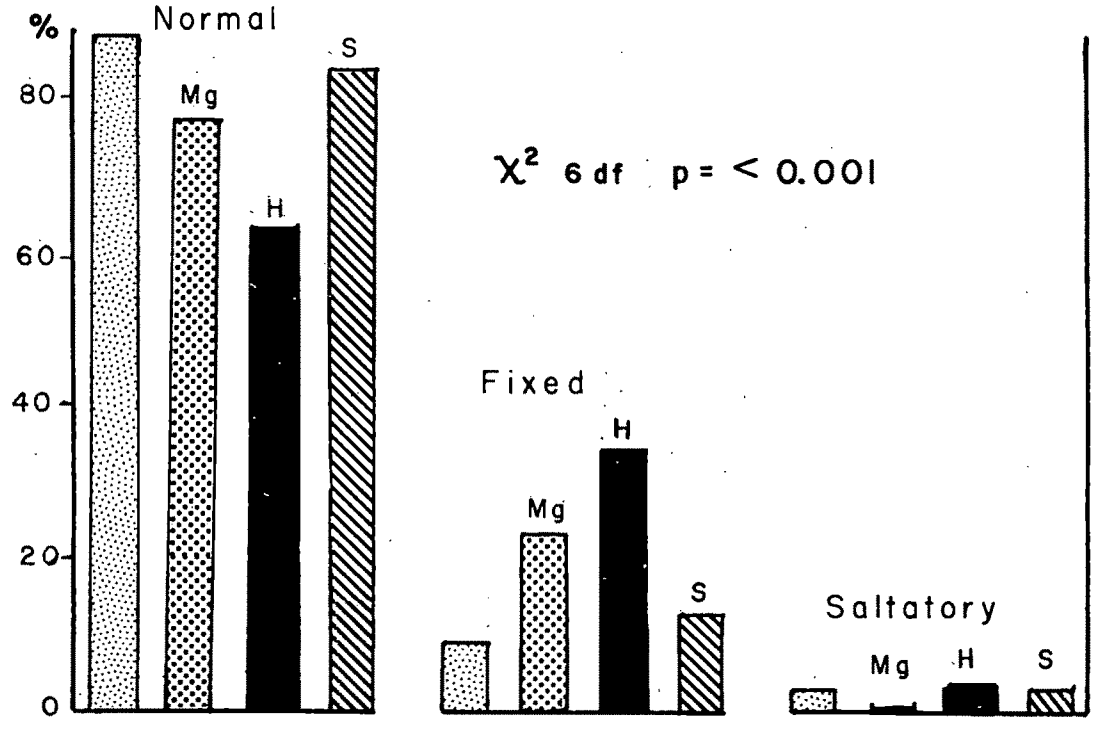


Fig. 2. Incidence of FHR baseline alterations associated with intrapartum administration of drugs. There are significant differences. *Unlabeled bar,* No medication; *Mg,* magnesium sulfate; *H,* hydralazine; *S,* sedatives.

Table IV. Drugs administered by group

Group	*No.*	*No drug*	*Magnesium sulfate*	*Hydralazine*	*Sedatives*	*Combination*
1	326	165 (51%)	51 (16%)	17 (5%)	83 (25%)	10 (3%)
2	253	87 (34%)	47 (19%)	28 (11%)	59 (23%)	32 (13%)
3	87	5 (6%)	14 (16%)	37 (43%)	11 (13%)	20 (23%)
TOTAL	666	257 (39%)	112 (17%)	82 (12%)	153 (23%)	62 (9%)

df 6; $p < 0.001$.

Table V. Nonstress test by hypertensive group

Group	*n*	*Reactive*	*Nonreactive*	*Equivocal*
1	100	91 (91%)	7 (7%)	2 (2%)
2	93	80 (86%)	9* (10%)	4 (4%)
3	32	21 (66%)	10† (31%)	1 (3%)
TOTAL	225	192 (85%)	26 (12%)	7 (3%)

df 4; $p < 0.005$.
*One neonatal death.
†Two neonatal deaths.

among the nine patients who had fetuses with nonreactive tracings.

A total of 47 infants (7%) among all the study groups (including cesarean sections) were depressed at birth (Apgar score ≤ 6 at 5 minutes). Among those who had intrapartum tracings, the incidence of late decelerations in the depressed group was significantly higher (24%) than in the group with Apgar scores ≥ 7 at 5 minutes (4%) ($p < 0.005$). There were no significant differences in baseline rate between the two infant groups. However, a higher percentage of infants with low Apgar scores did have fixed baselines compared with those with Apgar scores ≥ 7 (Table VI). These findings suggest that, as in normotensive pregnancies, the presence of abnormal FHR patterns can serve as a warning that the infant may be depressed at birth. The perinatal mortality rate was calculated for each group (Table VII) to assess the overall effect of the severity of hypertension on the fetus. A marked rise in perinatal mortality was seen as the severity of hypertension increased. The more severely hypertensive groups (2 and 3) accounted for 83% of all stillbirths and 69% of all neonatal deaths. Causes of death of the 16 infants were as follows: respiratory distress syndrome, seven; intracranial hemorrhages, eight; and sepsis, one. The perinatal mortality rate for all patients who were delivered at Chicago-Lying In Hospital in 1980 and 1981 was 43 per 1000; this included all deaths regardless of birth weight, and encompassed patients with a variety of high-risk conditions. There was one maternal death in the study group in a patient with eclampsia at 26 weeks' gestation who probably received an excessive dose of magnesium sulfate.

Comment

Intrapartum FHR patterns in term hypertensive pregnancies were previously reported by comparison with normotensive controls.[4] In this study a comparison was made among groups of hypertensive gravid women to evaluate the effect of the degree of hypertension on the FHR patterns. A trend of higher incidence of late decelerations was seen among group 3 patients than among those with lower blood pressures. However, as in normotensive pregnancies, the presence of late decelerations foretells difficulty for the fetus as demonstrated by the 24% of cases with Apgar scores ≤ 6 at 5 minutes that had late decelerations, significantly higher than among those with Apgar scores ≥ 7 (only 4%).

Higher blood pressure resulted in a trend toward an increasing percentage of fetuses who experienced intrapartum tachycardia. A relative fetal tachycardia during the third trimester has been reported to occur before the development of maternal hypertension.[12] A prospective analysis of the fetal heart rate from 15 to 38 weeks' gestation has shown a higher rate in those gestations in which hypertension ultimately developed.[13] However, no differences in the incidence of fetal tachycardia between hypertensive and normotensive gravid women at term has been previously reported.[4] The higher percentage of cases of fetal tachycardia with increasing blood pressure as shown in this study may not be a result of fetal distress but instead the higher incidence of preterm infants in the group 3 patients (Table I). This suggestion is supported by the observation that the incidence of fetuses with tachycardia was the same in infants with Apgar scores ≤ 6 and those with higher scores.

Table VI. FHR baseline changes and Apgar scores*

Apgar score at 5 min	*No.*	*Normal*	*Fixed*	*Saltatory*
≤6	22	13 (59%)	9 (41%)	0
≥7	408	337 (83%)	61 (15%)	10 (2%)
TOTAL	430	350 (82%)	70 (16%)	10 (2%)

2 *df*; $p = 0.006$.

*In 17 cases the quality of tracing did not permit classification.

Table VII. Perinatal mortality

Group	*No. of cases*	*Stillbirths*	*Neonatal deaths*	*‰*
1	326	2	5	21
2	253	4	5	36
3	87	6	6	138
TOTAL	666	12	16	42

Perinatal mortality in hospital, 1980 and 1981, 43/1000.

The presence of a fixed baseline appears to portend an unfavorable prognosis for the newborn, as has been previously reported in normotensive[14, 15] and hypertensive pregnancies.[16] The incidence of fetuses with fixed baseline increased significantly ($p < 0.03$) in parallel with the severity of the hypertension (Fig. 1). A significantly higher number of infants with Apgar scores ≤ 6 also displayed that pattern, compared with those with higher scores ($p < 0.006$). The finding that neonatal death occurred only among infants who had nonreactive antenatal tracings was probably of equal importance. Although this last finding was not significant, there was only a small number of infants to analyze; perhaps with a larger number statistical significance would have been achieved.

The intrapartum administration of magnesium sulfate or sedatives (meperidine, morphine) was found to have little effect on the FHR patterns. The incidence of fetal tachycardia was insignificantly higher among those patients who received magnesium sulfate but, as previously reported,[4] the tachycardia probably preceded the administration of the drug. The same pattern was observed among those patients who received sedatives. This finding reflects the previously shown[4] negligible effects of these drugs on FHR baseline frequency. Use of magnesium sulfate has been reported to increase,[17] decrease,[18] or have no effect[4] on FHR variability. The changes in variability, if any, caused by magnesium sulfate are probably transient as indicated by others[16, 18] because in this study only 23% of patients who received magnesium sulfate (164) had fixed baseline tracings (Fig. 2); the severity of hypertension was a more important factor in these patients than was the magnesium sulfate. Administration of hydralazine usually does not cause changes in FHR patterns unless maternal hypotension is induced, resulting in late decelerations. Patients who received hydralazine did have an increase in late decelerations and fixed baseline tracing (Fig. 2). This could have been related to the fact that the need to administer the antihypertensive agent increased as the severity of hypertension increased, as shown in Table IV.

The perinatal mortality rates for the study groups correlate well with previous publications[8, 19] that have shown a corresponding increase in perinatal mortality as the mean arterial pressures rise. The increasing stillbirth rate with increasing severity of hypertension, regardless of the cause, indicates that close attention must be paid during the antepartum period to the well-being of the fetus. The higher incidence of abnormal intrapartum FHR patterns, especially fixed baseline tracings or the presence of late decelerations, and the resultant increase in neonatal deaths in the more severely hypertensive patients indicates the need for extremely close monitoring of the fetus. It would be advisable to use a fetal scalp electrode and an intrauterine pressure catheter to obtain the most reliable monitoring tracing, and when abnormal FHR patterns are detected the fetal scalp pH should be assessed whenever possible.

The following conclusions can be made on the basis of these findings.

1. Continuous fetal heart rate monitoring, properly recorded and interpreted, may indicate the fetuses at higher risk in hypertensive conditions.
2. A fixed baseline and late decelerations seem to be the two best predictors of a depressed neonate. Neonatal death was associated only with nonreactive NST.
3. Nearly one half of patients with severe hypertension were delivered prematurely, and more than one third were delivered by cesarean section.
4. The overall incidence of cesarean section delivery

was 22%, many of which were repeat operations, whereas more than half the patients were delivered spontaneously.

5. Drugs commonly used in management of hypertension do not predictably affect FHR patterns unless they trigger maternal hemodynamic changes.
6. The perinatal mortality among hypertensive patients was similar to the overall perinatal mortality of the hospital. However, there was an eightfold increase in the severely hypertensive group.
7. Close antenatal and intrapartum surveillance may help to reduce the risks for both mother and infant.

REFERENCES

1. Cibils LA. The placenta and newborn infants in hypertensive conditions. AM J OBSTET GYNECOL 1974;118:256.
2. Lin C-C, Lindheimer MD, River P, Moawad AH. Fetal outcome in hypertensive disorders of pregnancy. AM J OBSTET GYNECOL 1982;142:255.
3. Hendricks CH, Brenner WE. Toxemia of pregnancy: relationship between fetal weight, fetal survival, and the maternal state. AM J OBSTET GYNECOL 1971;109:225.
4. Cibils LA. Clinical significance of fetal heart rate patterns during labor. VII. Hypertensive conditions. J Reprod Med 1981;26:471.
5. Peck T. Electronic monitoring evidence of fetal distress in high risk pregnancies. J Reprod Med 1980;24:103.
6. Schifrin BS, Lapidus M, Doctor GS, Leviton A. Contraction stress test for antepartum FHR evaluation. Obstet Gynecol 1975;45:433.
7. Slomka C, Phelan JP. Pregnancy outcome in the patient with a reactive nonstress test and a positive contraction stress test. AM J OBSTET GYNECOL 1981;139:11.
8. Friedman EA, Ed. Blood pressure, edema, and proteinuria in pregnancy. New York: Alan R Liss, 1976:123-51.
9. Naeye RL, Friedman EA. Causes of perinatal death associated with gestational hypertension and proteinuria. AM J OBSTET GYNECOL 1979;133:8.
10. Page EW, Christianson R. Influence of blood pressure changes with and without proteinuria upon outcome of pregnancy. AM J OBSTET GYNECOL 1976;126:821.
11. Reiss R, O'Shaughnessy RW, Quilligan TJ, Zuspan FP. Retrospective comparison of blood pressure course during pre-eclamptic and matched control pregnancies. AM J OBSTET GYNECOL 1987;156:894.
12. Dalton KJ, Shepherd JH. Fetal tachycardia and pregnancy hypertension. In: Sammour MB, Symonds EM, Zuspan FP, El-Tomi N, eds. Pregnancy hypertension. Cairo: Ain Shams University Press, 1982:151-8.
13. Dawson AJ, Dalton KJ, Newcombe RG. Baseline FHR from 15 to 38 weeks gestation in hypertensive and normotensive pregnancies. Br J Obstet Gynaecol 1985;92:60.
14. Paul RH, Sindan AK, Yeh SY, Schifrin BS, Hon EH. Clinical fetal monitoring. VII. The evaluation and significance of intrapartum baseline FHR variability. AM J OBSTET GYNECOL 1975;123:206.
15. Schifrin BS, Dame L. Prediction of Apgar Score. JAMA 1972;219:1322.
16. Ammala P, Kariniemi V. Short term variability of fetal heart rate during pregnancies complicated by hypertension. Br J Obstet Gynaecol 1983;90:705.
17. Petrie RH, Yeh S-Y, Murata Y, et al. The effect of drugs on fetal heart rate variability. AM J OBSTET GYNECOL 1978;130:294.
18. Babaknia A, Niebyl J. The effect of magnesium sulfate on fetal heart rate baseline variability. Obstet Gynecol 1978;51:2s.
19. Pritchard JA, MacDonald PC, Gant N. Williams' obstetrics. 17th ed. New York: Appleton-Century-Crofts, 1985.

Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis

Cynthia Chazotte, MD, Barbara Girz, DO, Mordecai Koenigsberg, MD, and Wayne R. Cohen, MD

Bronx, New York

We report a case of a large chorioangioma diagnosed prenatally with concomitant meconium peritonitis and hydrops fetalis in the second trimester. Spontaneous regression of the tumor occurred, associated with some resolution of the fetal hydrops and delivery near term with good neonatal outcome. (Am J Obstet Gynecol 1990;163:1180-1.)

Key words: Chorioangioma, meconium peritonitis, nonimmunologic hydrops

Chorioangioma is the most common primary tumor of the human placenta. Nonimmunologic hydrops fetalis is a serious fetal complication associated with this neoplasm.[1] We diagnosed a large chorioangioma by ultrasonography in the second trimester that was associated with meconium peritonitis and hydrops fetalis. Spontaneous resolution of the tumor coincident with some resolution of the hydrops occurred.

Case report

A 24-year-old woman (gravida 3, para 0-1-1-1), was referred for fetal ultrasonographic examination at 20 weeks' gestation because of maternal congenital heart disease. The mother was Rh-negative without antibodies. The VDRL test was negative, and there was no history suggestive of other TORCH infections. Previous pregnancies resulted in a spontaneous first-trimester abortion and a vaginal delivery of a healthy infant at 36 weeks' gestation.

Ultrasonography revealed fetal measurements consistent with gestational age, no identifiable anomalies, and normal amniotic fluid volume. The posterior placenta had a localized area of thickening, which was thought to possibly be a chorioangioma. Repeat ultrasonography at 23 weeks' gestation showed obvious hydramnios, with placental and fetal subcutaneous edema, ascites, and a highly echogenic intraabdominal region (Fig. 1). A 5 × 6.5 × 5.5 cm chorioangioma was recognizable on the fetal surface of the placenta. The patient elected to continue the pregnancy but declined to allow any diagnostic or therapeutic interventions.

Subsequent serial ultrasonographic examinations every 10 to 14 days showed gradual regression of the placental tumor and fetal subcutaneous edema, but persistent fetal ascites and echogenic regions. Nonstress tests performed semiweekly beginning at 29 weeks' gestation were always reactive. Delivery of a 3300 gm infant with Apgar scores of 7 and 8 at 1 and 5 minutes, respectively, occurred at 35 weeks' gestation after spontaneous rupture of membranes. There was no evidence of coagulopathy or anemia at birth. Postnatal ultrasonographic and x-ray examinations confirmed ascites and calcifications in the abdomen consistent with meconium peritonitis. There were no perforations seen on gastrointestinal series. The infant had a normal neonatal course including normal gastrointestinal function, with resolution of his ascites. He has had normal growth and development in the first year of life. The 620 gm placenta had a 5 × 5 cm soft, friable lesion on the fetal surface near the cord insertion. Histologic examination of this area was consistent with focal infarction of a preexisting chorioangioma.

Comment

Hydrops fetalis of any cause is generally associated with an adverse prognosis and often prompts early delivery or even induced abortion. Both chorioangioma and meconium peritonitis have been found in association with this entity.[1, 2] In this case both conditions could have contributed to the hydrops. However, although meconium peritonitis may explain the ascites and polyhydramnios, it is unlikely that it would cause subcutaneous or placental edema. It is not clear whether the presence of the chorioangioma and meconium peritonitis was coincidental. It is possible that

From the Departments of Obstetrics and Gynecology and Radiology, Albert Einstein College of Medicine, Bronx Municipal Hospital Center.

Received for publication April 27, 1990; accepted May 11, 1990.

Reprint requests: Cynthia Chazotte, MD, Department of Obstetrics and Gynecology, Bronx Municipal Hospital Center, Pelham Parkway South and Eastchester Road, Room 709, Bronx, NY 10461.

6/1/22421

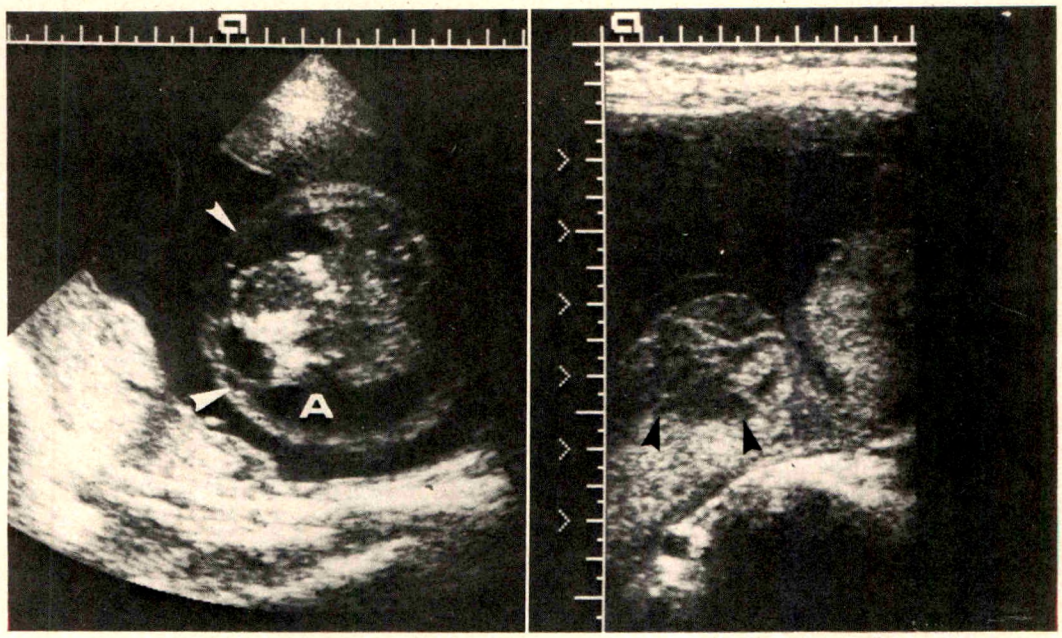


Fig. 1. *Left*, ultrasonogram of gravid uterus with transverse scan through fetal abdomen. There is evidence of fetal ascites *(A)* and subcutaneous edema *(arrows)*. Note echogenic regions on the intraabdominal viscera. There is also evidence of polyhydramnios. *Right*, ultrasonogram of posterior placenta near the cord insertion. Note heterogeneous region *(arrows)* consistent with chorioangioma. Fetal vein courses over tumor.

the meconium peritonitis may have been caused by a vascular thrombosis in the developing gastrointestinal tract, related to the chorioangioma. This possibility is supported by the fact that the tumor was a degenerative chorioangioma, a histologic variant frequently associated with intravascular thrombosis and infarction.

This case is significant in two respects. First, we observed the spontaneous infarction and gradual regression of a large chorioangioma with ultrasound imaging. Second, we demonstrated a partial resolution of pathologic fetal fluid associated with the regression of the angioma. Thus the prognosis for the hydropic fetus in the second trimester is not necessarily poor when a chorioangioma or meconium peritonitis has been identified.

REFERENCES

1. Maidman JE, Yeager C, Anderson V, et al. Prenatal diagnosis and management of non-immunologic hydrops fetal. Obstet Gynecol 1980;56:571-6.
2. Hutchison AA, Drew JH, Yu VHY, Williams ML, Fortune DW, Beischer NA. Non-immunologic hydrops fetalis: a review of 61 cases. Obstet Gynecol 1982;59:347-52.

Ovarian pregnancy: A report of twenty cases in one institution

Arieh Raziel, MD, Abraham Golan, MD, Mordechai Pansky, MD, Raphael Ron-El, MD, Ian Bukovsky, MD, and Eliahu Caspi, MD

Tel Aviv, Israel

A series of 20 cases of primary ovarian pregnancy that were diagnosed and treated in one institution is reported. The prevalence rate of 1:3600 deliveries seems to be increasing in past years and comprises 3.3% of all extrauterine pregnancies. Clinical presentation, possible pathogenesis, diagnostic steps, preferred management, and future fertility are detailed. Inasmuch as all our 18 fertile patients used an intrauterine contraceptive device before the operation, special emphasis is made on the controversial relationship between use of intrauterine contraceptive devices and ovarian pregnancy. (AM J OBSTET GYNECOL 1990;163:1182-5.)

Key words: Ovarian pregnancy

Primary ovarian pregnancy is one of the rarest types of extrauterine pregnancy. It is reported as 1:7000 to 40,000 deliveries and <3% of all ectopic gestations.[1-4] Because of the increased vascularity of the ovarian tissue, ovarian pregnancy usually is first seen with nonspecific signs and symptoms of ectopic pregnancy, often with hemoperitoneum that requires emergency surgery. With few exceptions, the suggestive initial diagnosis is made on the operating table and the final diagnosis only by histopathology on the basis of the four Spiegelberg criteria[5] establishing that the pregnancy is limited to the ovary and does not involve the tube. As such, great interest has always been focused on attempts to suggest the diagnosis of ovarian pregnancy *preoperatively*.[6] The evidence that the intrauterine contraceptive device (IUD) is involved in the pathogenesis of this rare but challenging condition continues to increase.[7-10]

In this study we report a series of 20 cases of primary ovarian pregnancy, all diagnosed and treated in one institution. The prevalence, presentation, possible pathogenesis, relation to the IUD, treatment, and results are discussed.

Material and methods

The medical records of 20 cases of ovarian pregnancy that occurred during a 19-year period (1971 to 1989) at Assaf Harofeh Medical Center, Zerifin, Israel, are reviewed. Pertinent data on each of the patients first seen before surgery are summarized in Table I.

From the Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Affiliated to Sackler School of Medicine, Tel Aviv University.
Received for publication January 30, 1990; accepted June 6, 1990.
Reprint requests: A. Raziel, MD, Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin, Israel 70300.
6/1/22988

Results

During these years there were 75,272 deliveries, of which 637 were ectopic pregnancies, comprising 0.85% of the total number of deliveries. A total of 21 cases (3.3%) of ectopic pregnancies were primary ovarian pregnancies for prevalence rate of 1:3600 deliveries. The prevalence is not constant but seems to be increasing, inasmuch as 10 cases occurred between 1971 to 1984 and 11 cases between 1985 to 1989, constituting a prevalence rate of 1:2100 in the past 5 years. One case that occurred in 1986 was included for prevalence calculation but was excluded from the series because of incomplete records. No particular past illness was reported. Four of the 21 patients had undergone abdominal surgery: three appendectomies and one explorative laparotomy after a road accident. A total of 90% of the women were multiparous with a mean age of 28 years (range, 20 to 34 years). The rate of associated use of IUD was extremely high, 18 of the 20 patients. The remaining two patients were nulligravid, one patient with 11-year primary infertility because of male factor and the second patient with 2 years of unexplained infertility. In fact, there was no case of ovarian pregnancy associated with a fertile woman who did not use an IUD. Signs and symptoms are detailed in Table II. Abdominal pain was present in almost all cases (95%) combined with menstrual irregularity (70%). Circulatory collapse occurred in six patients (30%). Other signs and symptoms are similar to those recorded in other tubal pregnancies. The mean duration from the last menstrual period to surgery was 6.3 weeks (range, 5 to 7.5 weeks). All cases since 1982 (13 of 20) had quantitative serum human chorionic gonadotropin β-subunit (β-hCG) determinations. In five patients the β-hCG level was <250 mIU/ml. The lowest value measured was 53 mIU/ml.

Table I. Patient's age, previous history, parity, and contraception history

Case no.	Year	Age	Past illness	Abdominal surgery	Parity	Contraception	
						Device	Duration (months)
1	1971	25	Rheumatic fever	—	2	IUD	6
2	1974	24	—	—	1	IUD	12
3	1975	30	—	Appendectomy	2	IUD	9
4	1975	34	Active asthma	—	4	IUD	24
5	1976	20	—	—	—	Infertility—unexplained	24
6	1976	24	—	—	2	IUD	18
7	1979	28	—	—	3	IUD	36
8	1982	32	—	—	3	IUD	12
9	1983	24.5	—	—	1	IUD	30
10	1984	30	—	—	1	IUD	4
11	1985	25	—	Laparotomy—road accident	2	IUD	24
12	1985	33	—	Appendectomy	3	IUD	12
13	1985	23	—	—	2	IUD	12
14	1986	24	—	—	2	IUD	36
15	1987	32	Hashimoto thyroiditis	—	2	IUD	54
16	1987	24	—	—	3	IUD	24
17	1988	33	—	—	—	Infertility—male factor	132
18	1988	28	—	Appendectomy	2	IUD	36
19	1989	33	—	—	3	IUD	6
20	1989	32	—	—	3	IUD	30

Surgical data including preoperative diagnosis, diagnostic procedures, operative diagnosis, and management are shown in Table III. Thirteen patients (65%) were suspected of having an ectopic pregnancy. Three (15%) were diagnosed as having a ruptured corpus luteum. Ovarian pregnancy was incidentally found in two cases: one at laparoscopy for suspected pelvic inflammatory disease and one at laparotomy for an acute abdomen not being aware of her pregnancy (before the era of β-hCG measurements). A Douglas puncture was performed in 12 of 20 cases (60%). Laparotomy followed a laparoscopic examination in nine patients (45%) and was directly performed without laparoscopy in 11 patients (55%). These laparotomies were preceded by a Douglas puncture in nine patients (82%). The ovarian pregnancy was equally distributed, 11 in the right ovary and nine in the left. Endometriotic implants were not found in any case. Estimated blood loss of ≥500 ml was found in 60% of patients. Blood transfusion was necessary in 35% of cases. Ovarian wedge resection was the most frequent surgical procedure performed (75%). In two of the patients who underwent salpingo-oophorectomy, tubal ligation was carried out for sterilization at the patients' preoperative request. Appendectomy was not frequently performed (15%). In no case was oophorectomy performed as a sole procedure. The final diagnosis was established by the histopathologic examination according to Spiegelberg's principles.[5]

Table II. Clinical presentations

Symptoms	Patients	%
Abdominal pain	19/20	95
Menstrual irregularity	14/20	70
Palpable adnexal mass	8/20	40
Circulatory collapse	6/20	30

Of the 18 fertile patients, four were lost to follow-up and six used various methods of contraception so that fertility could be followed up in only eight patients (40%). Of these, one had a tubal pregnancy and seven had uneventful intrauterine pregnancies.

Comment

An increase in the reported prevalence of ovarian pregnancies was published in recent years.[11, 12] Grimes reported on one ovarian pregnancy per 7000 deliveries and <3% of all ectopic pregnancies.[4] This corresponds well with our reported prevalence. It is not absolutely clear whether this is a true increase in prevalence or simply diagnosis of more ovarian pregnancies as a result of more awareness of this rare ectopic site, more tissue biopsy specimens from bleeding ovaries sent for histopathologic examination, and probably an early diagnosis of cases that would have otherwise resolved spontaneously, as happens in a certain proportion of tubal pregnancies.[13]

Table III. Diagnoses and surgical findings

Case no.	*Preoperative diagnosis*	*Diagnostic procedures*	*Operative diagnosis*	*Estimated blood loss*	*Surgical procedure*	*Blood transfusion*
1	EP	Laparoscopy	Right OP	350	Right WR + appendectomy	—
2	EP	—	Left OP	—	Left WR + appendectomy	—
3	EP	Laparoscopy + curettage	Right ruptured CL	Active bleeding	RSO	1
4	Acute abdomen	DP	Left OP	300	LSO + Pomeroy	—
5	Acute abdomen	DP	Right OP	2000	Right WR	4
6	EP	DP	Left OP	700	Left WR	2
7	EP	DP	Left OP	700	LSO + Pomeroy	2
8	Bleeding CL	DP	Left OP	2000	LSO + appendectomy	2
9	EP	DP	Right OP	Active bleeding	Right WR	—
10	EP	DP	Left OP	500	Left WR	—
11	Bleeding CL	DP	Right ruptured CL	1000	Right WR	2
12	PID	Laparoscopy + curettage	Right OP	400	Right WR	—
13	Left ovarian cyst	—	Right OP + Left ovarian cyst	150	Right WR + Left cystectomy	—
14	Bleeding CL	DP + laparoscopy	Right ruptured CL	1000	Right WR	—
15	EP	DP	Right OP	1500	Right WR	—
16	EP	DP + laparoscopy	Left OP	200	Left WR	—
17	EP	Laparoscopy	Right OP	200	Right WR	—
18	EP	Laparoscopy	Left OP	500	Left WR	—
19	EP	DP + laparoscopy	Right OP	100	Right WR	—
20	EP	Laparoscopy	Left OP	800	LSO	—

OP, Ovarian pregnancy; *DP,* Douglas puncture; *EP,* ectopic pregnancy; *CL,* corpus luteum; *PID,* pelvic inflammatory disease; *WR,* wedge resection; *LSO,* left salpingo-oophorectomy; *RSO,* right salpingo-oophorectomy.

Early quantitative serum β-hCG measurements, early pelvic ultrasonography, and particularly use of the vaginal probe showing "empty" uterus combined with symptomatology of ectopic pregnancy lead to more laparoscopies to find the site of ectopic gestation. An intact fallopian tube at laparoscopy with hemorrhagic ovary require a laparotomy and at least a wedge resection and hemostatic suturing.

The cause of primary ovarian pregnancy remains obscure. Boronow[14] concludes that chance is the reasonable explanation of ovarian pregnancies. Other hypotheses have suggested interference in the release of the ovum from the ruptured follicle, malfunction of the tubes, inflammatory thickening of the tunica albuginea, and parthenogenesis. In contrast to patients with tubal pregnancies, those with ovarian pregnancies very seldom have a history of pelvic inflammatory disease. In our series, no woman reported a past pelvic inflammatory disease, and at surgery we rarely found pelvic adhesions. Similar findings were reported by Sandevi et al.[10] and Chilanda,[11] but are in contradiction with Grimes et al.[4] who reported a previous reproductive system pathology or infertility in more than half their 24 cases.

The relationship between the IUD use and ovarian pregnancy is not new, but is very controversial. In 1970 Lehfeldt[15] conducted a survey of women who became pregnant while using an IUD and by simple mathematic extrapolation concluded that the IUD prevents implantation in the uterus with 99.5% effectiveness and in the tube with 99%, but that it has no effect on ovarian implantation. Grimes et al.,[4] in their literature overview until 1983, reported on ≤25% concurrent use of an IUD and ovarian pregnancy. Seventeen cases of 25 (68%) had an IUD in situ in Sandevi's recent study published in 1987.[10] In our series, 18 of 20 women (90%) had an IUD in situ when ovarian pregnancy was diagnosed, with an average duration of use of 2 years and a range of 6 to 54 months. The remaining two women were infertile before and remained so after

surgery. Thus all the fertile women in our series used an IUD at the occurrence of ovarian pregnancy. This fact strongly suggests that the IUD is somehow involved in the pathophysiology of ovarian pregnancy. Ovarian endometriosis does not seem to be an important causative factor, inasmuch as we found no evidence of the disease in any of our cases. This was also confirmed by others.[12]

Most ovarian pregnancies are symptomatic with abdominal pain being the most frequent sign along with menstrual irregularity. There was only one asymptomatic case in our series in which the ovarian pregnancy was associated with a contralateral torted ovarian cyst that could have masked the clinical presentation of the ovarian pregnancy.

The clinical presentation is usually more dramatic than that in tubal pregnancies. Because of the increased vascularity of the ovarian tissue and the growing gestation, rupture of the ovary and hemoperitoneum usually occur. Circulatory collapse may ensue rapidly (30% in our series) and even when the circulating system still holds, signs and symptoms of acute hemoperitoneum may become evident and immediate laparotomy is indicated, as in the majority of our patients. A Douglas puncture seems to be an important diagnostic tool. Hemoperitoneum was found in 95% of our cases with an average blood loss of 730 ml. Grimes et al.[4] reported that the prevalence of rupture among the ovarian pregnancies in his series was 91.6% and the average blood loss was 885 ml.

With the improvement of ultrasonographic skill and instrumentation, especially the vaginal probe, ovarian pregnancy has been recently demonstrated preoperatively.[16, 17] A live embryo within the ovary was demonstrated in one of our cases (no. 8), but because of clinical signs of shock there was no time for ultrasonographic examination. Despite the limited aid of ultrasonography in preoperative diagnosis in this series, we believe that vaginal ultrasonography may potentially be a very helpful diagnostic tool.

No case of repeat ovarian pregnancy has been reported in contrast to the approximate 15% recurrent tubal pregnancy rate.[18] In contrast to the tube, rupture of the ovary is a frequent phenomenon occurring periodically at ovulation. In view of this and the fact that seven intrauterine pregnancies were reported among the eight fertile patients after surgery, we agree with Portuondo[19] and others who believe that fertility after an ovarian pregnancy remains unmodified.

REFERENCES

1. Hertig AT. Discussion of Gerin-Lajoie L. Ovarian pregnancy. Am J Obstet Gynecol 1951;62:920.
2. Golan A, Menchovsky E, Edelstein T. Primary ovarian pregnancy and the intrauterine contraceptive device. S Afr Med J 1977;52:1130.
3. Ellis RW. Ovarian pregnancy. Obstet Gynecol 1978;15:54.
4. Grimes HG, Nosal RA, Gallagher JC. Ovarian pregnancy: a series of 24 cases. Obstet Gynecol 1983;61:174.
5. Spiegelberg O. Zur casuistik der ovarialsch wangerschaft. Arch Gynaekol 1878;13:73.
6. Campbett JS, Hacquebard S, Milton DM, Hurteans GD, Borda ST, Sirris J. Acute hemoperitoneum, IUD and occult ovarian pregnancy. Obstet Gynecol 1974;3:438.
7. Vessey MP, Johnson B. Outcome of pregnancy in women using an IUD. Lancet 1974;1:495.
8. McMorries K, Lofton RH, Stinson JC. Is the IUD increasing the number of ovarian pregnancies? Contemp Obstet Gynecol 1979;13:165.
9. Gray CL, Ruffolo EH. Ovarian pregnancy associated with intrauterine devices. Am J Obstet Gynecol 1978;132:134.
10. Sandvei R, Sandstad E, Steier JA, Ulstein M. Ovarian pregnancy associated with the intrauterine contraceptive device. Acta Obstet Gynecol Scand 1987;66:137.
11. Chilanda GS, Lev-Gur M, Kim ES, Greston WM, Stern W, Kleiner G. Primary ovarian pregnancy two years after bilateral tubal ligation. J Reprod Med 1978;32:697.
12. Hallatt JG. Primary ovarian pregnancy: a report of twenty-five cases. Am J Obstet Gynecol 1982;143:55.
13. Lund JJ. Ectopic pregnancy. Br J Obstet Gynaecol 1955;62:1.
14. Boronow RC, McElin TW, West RH, Buckingham JC. Ovarian pregnancy: report of four cases and thirteen-year survey of the English literature. Am J Obstet Gynecol 1965;91:1095.
15. Lehfeldt H, Tietze C, Gorstein F. Ovarian pregnancy and the intrauterine device. Am J Obstet Gynecol 1970;108:1005.
16. Russell JB, Cutler LR. Transvaginal ultrasonographic detection of primary ovarian pregnancy with laparoscopic removal: a case report. Fertil Steril 1989;51:1055.
17. Malinger G, Achiron R, Treschan O, Zakut H. Ovarian pregnancy—ultrasonographic diagnosis. Acta Obstet Gynecol Scand 1988;67:561.
18. Langer R, Bukovsky I, Herman A, Sherman D, Sadovsky G, Caspi E. Conservative surgery for tubal pregnancy. Fertil Steril 1982;38:427.
19. Portuondo JA, Ochoa C, Gomez BJ, Uribarren A. Fertility and contraception of 6 patients with ovarian pregnancy. Int J Fertil 1984;29:254.

Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21

Norman Ginsberg, MD, Alan Cadkin, MD, Eugene Pergament, MD, PhD, and Yury Verlinsky, PhD

Chicago, Illinois

Biparietal diameter/femur length ratio and nuchal thickness were found to be sensitive indicators for the prenatal detection of trisomy 18 and trisomy 21. A biparietal diameter/femur length ratio >1.5 SD above the control mean correctly identified 5 of 11 (46%) fetuses with trisomy 21 and 3 of 4 (75%) fetuses with trisomy 18. Nuchal thickening (6 mm or more) correctly identified 5 of 12 (41%) fetuses with trisomy 21 and 2 of 4 (50%) fetuses with trisomy 18. The sensitivity and specificity of the biparietal diameter/femur length ratio in detecting either aneuploidy was 53% and 93%, respectively, whereas a thickened nuchal fold had a sensitivity of 44% and a specificity of 100%. The combined use of the two ultrasonographic measurements had an overall sensitivity of 81% and a specificity of 93%. Prospective ascertainment of these two trisomies appears warranted in low-risk populations. (Am J Obstet Gynecol 1990;163:1186-90.)

Key words: Trisomy 18, trisomy 21, ultrasonographic screening, fetus

Prenatal genetic studies to detect chromosomally abnormal conceptions have generally been limited to women of advanced maternal age and those who have experienced a previous conception with a chromosomal aberration. However, it is young mothers with completely normal pregnancy histories who subsequently are delivered of the largest number of chromosomally abnormal newborns. Although their individual risk for such an occurrence is very small, women younger than 34 years of age have >80% of all affected pregnancies. For this reason less than 20% of all trisomy 21 pregnancies could potentially be detected prenatally by means of chorionic villus sampling or amniocentesis.[1]

An important benefit to the screening of pregnancies for open neural tube defects is the recognition that a low concentration of maternal serum α-fetoprotein (AFP) may be associated with trisomy 21.[2] Critical assessment of the effectiveness of these screening programs reveals their disappointing low sensitivity (12% to 21%) as well as high false-positive rate (7% to 9%).[2, 3]

Recently the combination of maternal serum AFP, β-subunit human chorionic gonadotropin, and serum estriol have shown promise for greater detection of Down syndrome than maternal serum AFP alone.[4-6]

From the Reproductive and Medical Genetics Section, Department of Obstetrics and Gynecology, Illinois Masonic Medical Center.
Received for publication August 7, 1989; revised March 9, 1990; accepted June 2, 1990.
Reprint requests: Norman Ginsberg, MD, 30 N. Michigan Ave., Chicago, IL 60602.
6/1/22875

It has been reported that biparietal diameter/femur length ratio and nuchal skin thickness measurements can identify 50% to 70% of trisomy 21 pregnancies in the second trimester,[7, 8] but this has been disputed.[9, 10] This study confirmed that a combination of both measurements may be the most sensitive, noninvasive approach to the prenatal identification of trisomy 21 and can be successfully applied to the detection of trisomy 18 as well.

Material and methods

The ultrasonographic equipment used in this study consisted of an ATL Ultramark-4, Diasonics Wide Vu, and Acuson-128. All transducers were 3.5 mHz with a combination of arc sector and linear array.

The study group consisted of 12 cases of trisomy 21 and four cases of trisomy 18 previously diagnosed by amniocentesis. The control group comprised 212 randomly selected cases with a normal karyotype as determined by chorionic villus sampling or amniocentesis. The control group was used to establish a gestational curve for the biparietal diameter/femur length ratio. The control group biparietal diameter/femur length ratio then became the basis for comparison against the trisomic fetuses. The criteria for inclusion into the control group were either a first-trimester ultrasonographic evaluation confirming gestational age (determined by crown-rump length), or a second-trimester ultrasonographic evaluation confirming that the gestational age did not vary from the stated menstrual age by more than 1.5 weeks (determined by biparietal diameter and femur length measurements).[11, 12] All pa-

tients who underwent chorionic villus sampling had a similar ultrasonographic evaluation as those undergoing amniocentesis.

The biparietal diameter was measured with electronic calipers from leading edge to leading edge at the level of the thalami. The femus was measured in a plane that visualized the greater trochanter to the end of the ossified shaft. When there was a question regarding the exact placement of the markings by the electronic calipers, the acoustical shadow created by the ossified bone was used. For each of the trisomies the number of cases with a biparietal diameter/femur length ratio >1.5 SDs above the mean of the control for a given gestational age was determined. The selection of 1.5 SD above the mean of the controls was used to confirm the work of Lockwood et al.[7] as well as to check its applicability to trisomy 18.

The nuchal thickness was measured at both the level of the occiput, at the midpoint of the cerebellum in the transverse plane, as well as in the dorsal midsagittal plane at the same level, and was considered abnormal if >6 mm. One case of trisomy 21 was not included in the biparietal diameter/femur length analysis because the length of the femur was not measured in the original ultrasonographic evaluation; it was included in the analysis of nuchal thickness.

All abnormal cases were independently evaluated from videotapes by two of the authors (N. G. and A. C.) to assure measurement accuracy. Any differences in measurement were reevaluated and based on this review. Two femur length measurements were changed and one previously undiagnosed nuchal thickening was detected. The control group was also reviewed and no changes were made.

Results

The control value data are summarized in Table I. A biparietal diameter/femur length ratio >1.5 SDs above the control population mean was present in five of 11 (46%) fetuses with trisomy 21 and three of four (75%) fetuses with trisomy 18; there were 14 fetuses (6.6%) whose biparietal diameter/femur length ratios exceeded 1.5 SDs from their respective ratio means in the control population.

Five of the 12 (41%) fetuses with trisomy 21 and two of four (50%) fetuses with trisomy 18 had thickened (>6 mm) nuchal folds. There were no cases of occipital nuchal thickening ≥6 mm in the 212 fetuses that comprised the normal population.

If the data on the biparietal diameter/femur length ratio are combined with those of fetuses with a thickened nuchal fold nine of 12 (75%) fetuses with trisomy 21 and all four fetuses with trisomy 18 are identified, with a false-positive rate of 6.6%.

Table I. Control value for normal biparietal diameter/femur length ratio

Gestational age (wk)	*Normal biparietal diameter/femur length ratio (mean + 1.5 SD)*
14	1.96
15	1.83
16	1.72
17	1.66
18	1.65
19	1.63
20	1.64

The sensitivity (true-positive results) and specificity (true-negative results) of the two ultrasonographic measurements in the detection of either aneuploidy of chromosome 18 or 21 are summarized in Table II. The sensitivity and specificity of the biparietal diameter/femur length ratio was 53% and 93%, respectively. A thickened nuchal fold had a sensitivity of 44% and a specificity of 100%. The combined use of the two ultrasonographic measurements had an overall sensitivity of 81% and a specificity of 93%.

Comment

The biparietal diameter/femur length ratio and occipital nuchal thickness measurements can be used to detect a significant number of trisomic fetuses. When the biparietal diameter/femur length ratio with a 1.5 SD cut-off was applied, 75% of the fetuses with trisomy 18 were detected. A nuchal thickening of ≥6 mm was present in 50% of the fetuses with trisomy 18. By combining the use of these ultrasonic measurements, all four of these chromosomally abnormal fetuses were identified between 14 and 17 weeks' gestation.

The results of this study demonstrated remarkable agreement with those previously reported by Lockwood et al.[7] and Benacerraf et al.[8] despite the fact that each study established different normal values for the biparietal diameter/femur length ratio for each week of gestation. This strengthened the recommendation that each investigator needs to establish his or her own set of normal biparietal diameter/femur length ratios, which is dependent on their ultrasonographic equipment and their measurement techniques.[7]

The value of nuchal thickening in identifying fetuses with trisomy 21 in the second trimester of pregnancy has been questioned.[9] In this study a nuchal thickening of ≥6 mm was uniformly associated with aneuploidy and, in contrast to the previous report to Toi et al.,[9] there were no false positives in the control group. Moreover, the demonstration of nuchal thickening was clearly more indicative of the presence of a chromosomal abnormality than was a biparietal diameter/femur length ratio >1.5 SDs from the control mean. Mea-

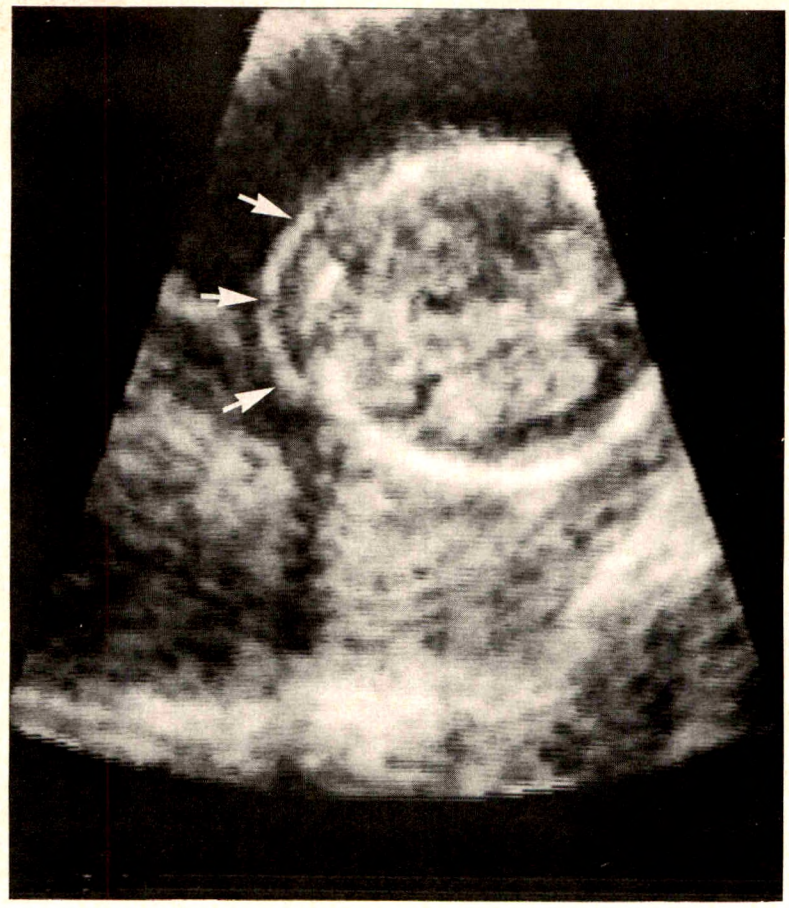


Fig. 1. Nuchal thickening at the level of the cerebellum. *Arrows* indicate skin line wrapping around parietal temporal complex.

Table II. Distribution of normal and abnormal biparietal diameter/femur length ratios and nuchal thickness for aneuploid (trisomies 18 and 21) and normal fetuses

Sign	*Aneuploid fetuses*	*Normal fetuses*
Biparietal diameter/femur length		
Abnormal*	8	14
Normal	7	198
	(Sensitivity 53%)	(Specificity 93%)
Nuchal thickness		
Abnormal†	7	0
Normal	9	212
	(Sensitivity 44%)	(Specificity 100%)
One or more signs		
Abnormal	13	14
Normal	3	198
	(Sensitivity 81%)	(Specificity 93%)

*Ratio value > 1.5 SDs from normal mean.
†Nuchal thickness > 6 mm.

surement was taken in a plane at the level of the occiput, as described by Benacerraf et al.[13] from the outer skin line to the outer edge of the occipital bone. A thickened curvilinear extension of the skin line toward the level of the convexity of the parietal temporal complex (Fig. 1) associated with the abnormal thickening. This curvilinear line should not be confused with the head abutting against the amniotic membrane, particularly when there is an ultrasonographically residual chorioamniotic space. If there was any question as to whether

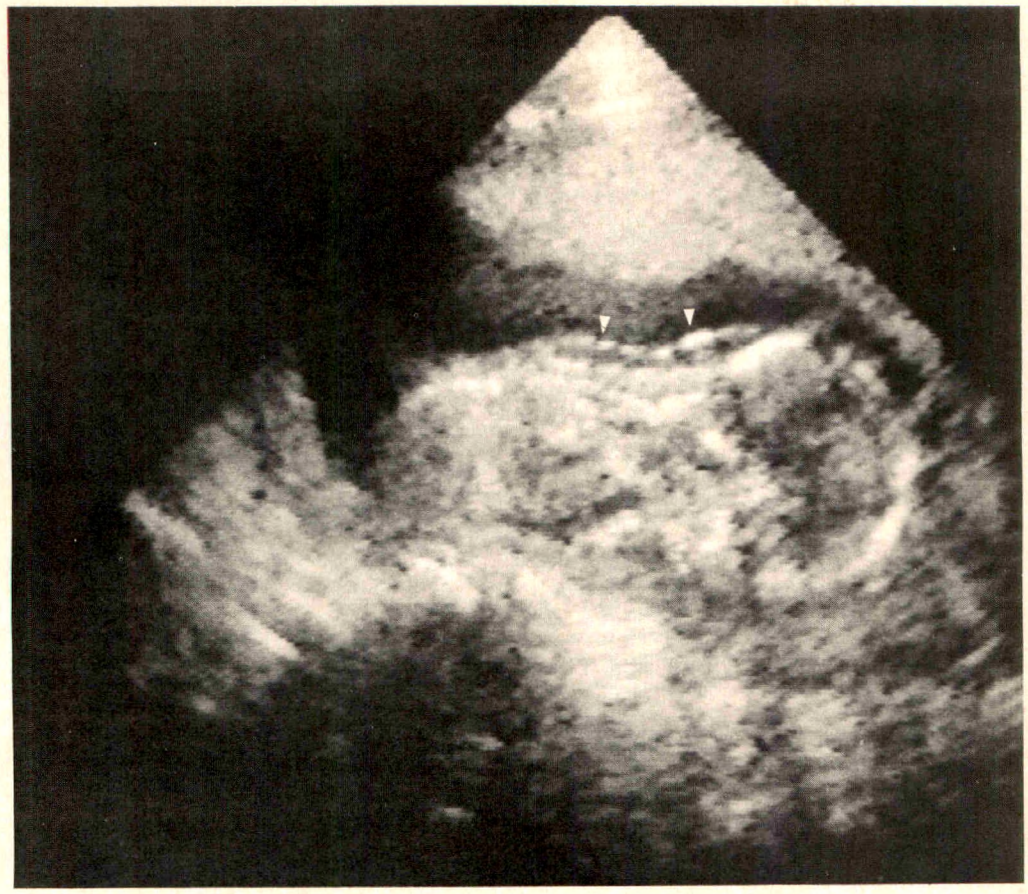


Fig. 2. Nuchal thickening in the dorsal midsagittal plane. *Arrows* point to skin line. Hypoechoic area represents areolar edema.

the measured thickening was actually present, the fetus was examined in the dorsal midsagittal plane (Fig. 2). When the angle of the transducer was caudal to a line drawn from the lower margin of the orbit to the auricular point and extended to the center of the occipital bone (Reid's baseline), this plane ruled out any false-positive findings. The fetus, when viewed in the dorsal midsagittal plane with high-resolution ultrasonographic equipment, usually demonstrated a double line in the region of the nuchal thickening. This skin line was a hypoechoic stripe that represented edematous areolar tissue; below this the muscles of the neck were seen. This hypoechoic area added to the thickening in the nuchal region. With the head in normal flexion, the skin bulges at the occiput even if the double line cannot be seen in the dorsal midsagittal plane.

It has been suggested that all pregnant women be screened during the second trimester for trisomy 21,[8] although caution has been urged in making such a practice widespread.[14]

The positive predictive value of biparietal diameter/femur length ratio and nuchal thickness is an estimate of the probability that a fetus identified by these ultrasonic measurements as having a high risk of trisomy 21 or 18 actually is aneuploid for one of these chromosomes. The general population incidence for trisomy 21 and 18 at term is 1 per 1000 and 1 per 7407, respectively, whereas the fetal loss rate between the second trimester and term is 29% and 67%, respectively.[15, 16] The resulting positive predictive value in this study would be 1/47. Thus 46 amniocenteses on chromosomally normal gestations would be necessary for each diagnosis of either trisomy 18 or 21. Amniocentesis currently is performed for advanced maternal age when the risk of a live-born infant with trisomy 21 is 1/385[15] and for a low maternal serum AFP value when the risk of a second-trimester gestation with trisomy 21 is 1 per 250.[17] Therefore about one eighth to one third as many amniocenteses generated from ultrasonographic screening evaluations are required to detect either trisomy 18 or 21 compared with advanced maternal age and low maternal serum AFP level. Ultrasonography is noninvasive and widely available in the United States; such screening would not require extensive technical training or the use of overly expensive ultrasonographic equipment. Finally, we estimate that

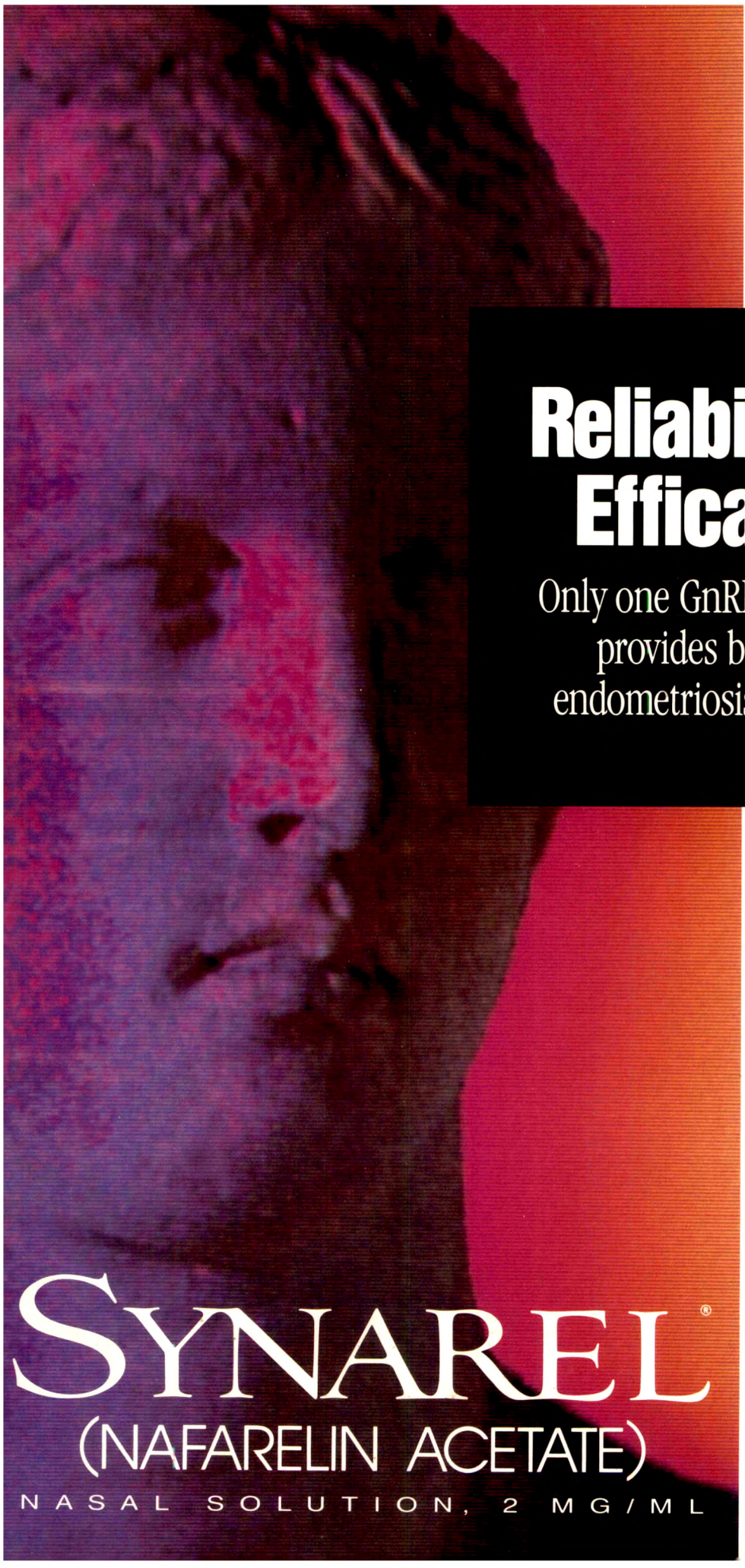
a program of ultrasonographic screening and follow-up amniocentesis in a low-risk population would cost approximately $150,000 to detect one case of trisomy 18 or 21, or about $100,000 less than the cost per case when amniocentesis is performed for advanced maternal age or low maternal serum AFP level.

If the approach described in this article were to be widely applied, it might be possible to considerably reduce the number of undiagnosed trisomies before delivery. This approach may be particularly helpful in cases with a low maternal serum AFP level to decide the most appropriate management plan.

Prospective ascertainment of trisomies 18 and 21 now appears warranted to determine if ultrasonographic evaluation would effectively predict chromosomally abnormal gestations in low-risk populations. The possibility that trisomy 13 gestations might also be identified by ultrasonographic assessment of biparietal diameter/femur length ratio and nuchal thickness should likewise be investigated.

REFERENCES

1. Adams M, Erickson J, Leyde P, Oakley G. Down's syndrome: recent trend in the United States. JAMA 1981; 246:758-60.
2. Cuckles HS, Walk NJ, Lindebaum RH. Maternal serum α-fetoprotein measurement: a screening test for Down's syndrome. Lancet 1984;1:926-9.
3. Spencer K, Carpenter P. Screening for Down's syndrome using α-fetoprotein: a retrospective study indicating caution. Br Med J 1985;290:1940-3.
4. Canick JA, Knight GJ, Palomaki GE, et al. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J Obstet Gynaecol 1988;95:330-3.
5. Petrocik E, Wassman ER, Kelly JC. Prenatal screening for Down syndrome with maternal serum human chorionic gonadotropin levels. Am J Obstet Gynecol 1989;161: 1168-73.
6. Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Pernat Diagn 1987; 7:623-30.
7. Lockwood C, Benacerraf B, Krinsky A, et al. A sonographic screening method for Down syndrome. Am J Obstet Gynecol 1987;157:803-8.
8. Benacerraf BR, Gelman R, Frigoletto FD. Sonographic identification of second trimester fetuses with Down's syndrome. N Engl J Med 1987;317:1371-5.
9. Toi A, Simpson GF, Filly RA. Ultrasonically evident fetal nuchal skin thickening; is it specific for Down syndrome? Am J Obstet Gynecol 1987;156:150-3.
10. Lynch L, Berkowitz GS, Chitkara U, Wilkins IA, Megakej KE, Berkowitz RL. Ultrasound detection of Down syndrome: is it really possible? Obstet Gynecol 1989;73:267-70.
11. Sabbagha RE, Barton BA, Barton BF, Kingas E, Orgill J, Turner JH. Sonar biparietal diameter. II. Predictive of three fetal growth patterns leading to a closer assessment of gestational age and neonatal weight. Am J Obstet Gynecol 1976;126:485-90.
12. Jeanty P, Rodesch F, Delbeke D, Dumont JE. Estimation of gestational age from measurements of fetal long bones. J Ultrasound Med 1984;3(suppl 2):75-9.
13. Benacerraf B, Frigoletto F, Cramer D. Down syndrome: sonographic sign for diagnosis in the second-trimester fetus. Radiology 1987;163:81-3.
14. Elias S, Annas GJ. Routine prenatal genetic screening. N Engl J Med 1987;317:1409-10.
15. Hook EB, Cross PK. Schreinemacher DM. Chromosonal abnormality rates at amniocentesis and in live-born infants. JAMA 1983;249:2034-8.
16. Hook EB. Chromosome abnormalities and spontaneous fetal death following amniocentesis: further data and association with maternal age. Am J Hum Genet 1983; 35:110-6.
17. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984;148:886-94.



Reliability & Efficacy:

Only one GnRH* agonist provides both in endometriosis therapy

SYNAREL®

(NAFARELIN ACETATE)

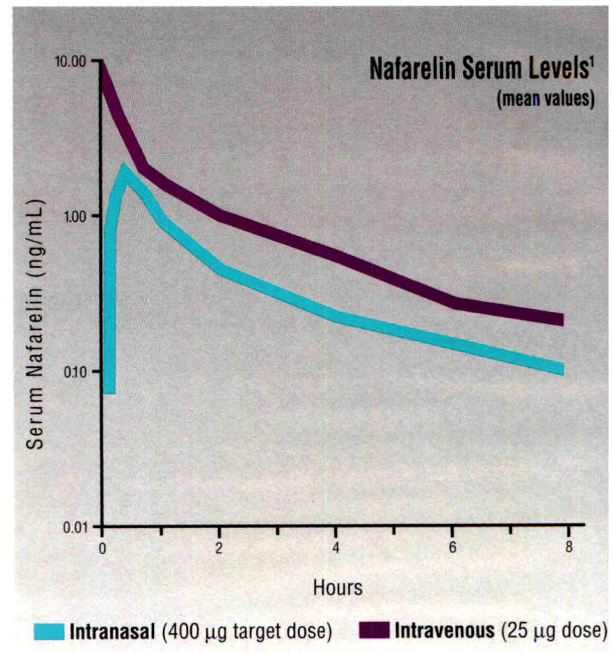
NASAL SOLUTION, 2 MG/ML

*Gonadotropin-releasing hormone analog.
Please see brief summary of prescribing information on last page.

The Only GnRH* Agonist That Satisfies The Needs Of Endometriosis Therapy



Reliable Dosing Control



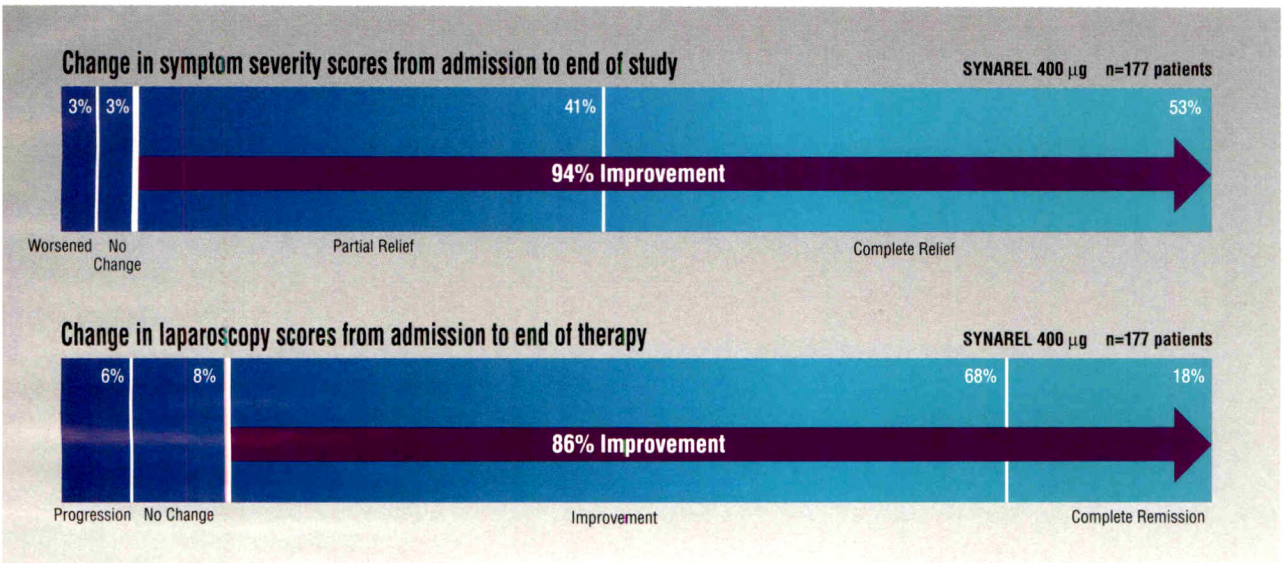
B.I.D. controlled-dose administration offers unique clinical benefits:

- Controlled intranasal administration
- Highly accurate metered-dose intranasal delivery
- Proven absorption through nasal mucosa
- Well tolerated, with a low incidence of nasal irritation (10%)

Intranasal administration produces nafarelin serum levels comparable to I.V. dosing

- In pharmacokinetic studies, SYNAREL wa rapidly absorbed into systemic circulatior following intranasal administration
- Maximum serum concentrations were achieved between 10 and 40 minutes with intranasal administration.[1]

Outstanding Efficacy In The Management Of Endometriosis, An Estrogen-Dependent Disease



94% of Synarel patients reported improvement of symptoms

- Proven to relieve dysmenorrhea, dyspareunia, and pelvic pain
- Efficacy sustained in 83% of patients at six months posttreatment
- Pregnancy should be ruled out before therapy with SYNAREL is initiated. Patients should be advised to use a nonhormonal method of contraception when taking SYNAREL.

86% of Synarel patients showed improvement of endometriotic lesions

- Improvement in all stages of endometriosis confirmed by post-laparoscopy scores†
- Retreatment cannot be recommended since safety data for retreatment are not currently available



SYNAREL®

(NAFARELIN ACETATE)

NASAL SOLUTION, 2 MG/ML

Endometriosis therapy breakthrough

Please see brief summary of prescribing information on last page.

yntex document #829-205-A.

onadotropin-releasing hormone analog.

ased on American Fertility Society
iparoscopy score stages: (absent, score
); I (minimal, 1–5); II (mild, 6–15); III
noderate, 16–40); IV (severe, 40+).

The Only GnRH* Agonist Indicated For The Management Of Endometriosis

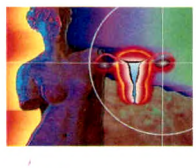
- Level of serum estradiol suppression proven effective for outstanding improvement of endometriotic symptoms and lesions
- B.I.D. controlled-dose intranasal administration facilitates patient management in the event of adverse reactions (The most frequent side effects are those associated with hypoestrogenism.)
- Convenient treatment regimen for patients and physicians
- Eligible for reimbursement as an indicated therapeutic option for endometriosis

For more information, please call:
1-800-727-0400



*Gonadotropin-releasing hormone analog.

© 1990 Syntex Laboratories, Inc.
829-H2-205-90



SYNAREL®
(NAFARELIN ACETATE)
NASAL SOLUTION, 2 MG/ML

Endometriosis therapy breakthrough

BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chlorid glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and olde treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undia nosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL ma cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patien becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women wh are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pre nancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contr ception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal co traception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnar during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient shoul notify her physician if regular menstruation persists. Patients missing successive doses may experience breakthroug bleeding.

2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or ar allergic to any of its ingredients.

3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should b used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consu her physician.

4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritatio occurred in about 10% of patients.

5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of thi loss may not be reversible. During one six-month treatment period, this should not be important. In patients with majc risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history c osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNARE may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatmer with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content.

6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topic nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decreas the possibility of reducing drug absorption.

7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetat is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound t plasma proteins at 4°C; therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. No mal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitar gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may b misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats an mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dos respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes c administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonge periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidenc of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There wa an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treate groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there wa a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose female No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive t hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potenti

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment wa discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rat throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on th relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/8 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 60 µg/kg/day and 0.18 µg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administratio throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fet weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted i human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determine SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. I controlled studies comparing SYNAREL (400 µg/day) and danazol (600 or 800 mg/day), the following adverse rea tions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19% 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20% myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8% 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depressio (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticari asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be po sibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mas measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, con pared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bon density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mas measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebra mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if an decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than th recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause adc tional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twic the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and level returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 µg/day and 2% on danazol had tota cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had pos treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8 mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment value were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of th patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) com pared with 4.3% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fraction (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients o SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patient SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

February 1990

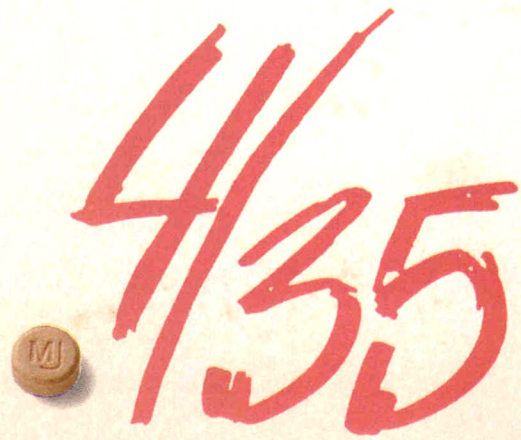
© 1990 Syntex Laboratories, Inc.

Committed to the OB/GYN and women's healthcare

SYNTEX

SYNTEX LABORATORIES, INC
PALO ALTO, CA 94304

WE HAVE A POINT TO MAKE.



SO LITTLE CAN DO SO MUCH.

The Smart Start

OVCON® 35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Ovcon® 35 offers your patients contraception, with the lowest norethindrone dose of any combination OC. Low incidence of both androgenic side effects[1] and breakthrough bleeding.[2]

Ovcon 35 has a unique estrogen/progestogen ratio. And remember—Ovcon 35 has no generic equivalent.

Amazing how so little can do so much. Our point. Exactly.

1. Data on file, Bristol-Myers Company.
2. Gordon M, Boria M: Multiclinic study of a low-dose oral contraceptive, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive.* New York, Biomedical Information Corp., 1981, pp 8-11.

Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.

Dedicated to Women's Health Care

Please see next page for brief summary of complete prescribing information.

C-K39-1989

© 1989, Bristol-Myers Company, Evansville, Indiana 47721 U.S.A.

OVCON® 35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptive or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000; ages 35-44, 33/100,000; ages 45-49 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were age 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. (2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledemia; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use In Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstibestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) *Gallbladder Disease.* Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Headaches.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. (11) *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) *Breast-Feeding.* A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norephinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein -bound iodine (PBI), T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG, free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, hemolytic uremic syndrome, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erhythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.

C-K39-1989

© 1989, Bristol-Myers Company, Evansville, Indiana 47721 U.S.A.

Second-trimester ultrasonography: Determinants of visualization of fetal anatomic structures

Val Catanzarite, MD, PhD,[a] and J. Gerald Quirk, MD, PhD[b]
San Diego, California, and Little Rock, Arkansas

Little information has been published regarding rates of visualization of fetal anatomic structures or factors affecting visualization in unselected patients. We prospectively studied these points by scoring visualization of intracranial, spinal, cardiac, ventral wall, umbilical cord, stomach, bladder, and genital anatomy in 573 consecutive midtrimester scans in which no fetal anomalies were seen. Each feature was scored 0, 1, or 2, corresponding to poor, adequate, or optimal visualization, and these eight numbers were summed for an overall visualization score. Overall visualization fell abruptly beyond maternal weight 165 pounds ($p < 0.001$) and rose with advancing gestational age ($p < 0.001$). Placental location did not significantly influence visualization. Overall, an adequate screening scan, defined as adequate visualization of central nervous system, heart, stomach, ventral wall, bladder, and cord was obtained in 51% of scans, and optimal visualization of each of these in only 9% of cases. Factors that might improve anatomic visualization and the implications of these results for the use of ultrasonography in screening for congenital anomalies are discussed. (AAM J OBSTET GYNECOL 1990;163:1191-5.)

Key words: Second trimester, ultrasonography, fetal anatomy, fetal anomalies

The past decade has witnessed a veritable revolution in ultrasonographic technology, recognition of fetal anatomic abnormalities, and the application of ultrasonography to obstetric practice. In the past ultrasonographic examinations were performed primarily for evaluation of gestational age, fetal growth, amniotic fluid volume, and placental location[1, 2]; screening for anatomic defects was a secondary goal. With the current generation of abdominal ultrasonographic equipment, the fetal cortex and ventricles, cerebellum, choroid plexus, spine, stomach, kidneys, and heart can be clearly imaged during the early second trimester; for many fetal organs, not only visualization but also normal size ranges have been reported from 12 to 16 weeks' gestation onward. Second-trimester ultrasonography is now often performed as a screen for congenital anomalies,[3-5] and various views of the fetus have been advocated for incorporation into routine "screening" scans.[6, 7]

Most of the published literature addresses the issue of how early fetus structures can be seen with ultrasonography under ideal conditions rather than how often these structures are adequately visualized in practice. To address this issue we prospectively studied visualization of anatomic features of the fetus during second-trimester ultrasonography by two experienced examiners. In addition to defining frequencies of visualization, we tested the following specific hypotheses:

1. Anatomic visualization improves with advancing gestational age and decreases with increasing maternal weight and abdominal wall fat thickness.
2. Visualization is decreased in the presence of an anterior placenta.
3. Visualization of caudal structures is worse when the fetus is in breech presentation, and visualization of rostral structures is worse when the fetus is in vertex presentation.

Subjects and methods

The study population was drawn from patients who had second-trimester, transabdominal level II examinations performed by one of us in our genetics-ultrasound unit between July 1, 1988, and March 31, 1989. Patients were excluded if they were referred because of identified or suspected fetal anomalies, if the amniotic fluid volume was abnormally increased or reduced, or if fetal anomalies were identified during the scan.

All examinations were performed using an ATL/ADR Ultramark-4 ultrasound machine (Advanced Technology Laboratories, Bothell, Wash.) equipped with 5 MHz linear, 5 MHz annular, and 3 MHz linear and sector transducers. Examination began with recording of fetal number, lie, and presenta-

From The Perinatal Center, San Diego,[a] and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Arkansas for Medical Sciences.[b]
Received for publication June 30, 1989; revised May 21, 1990; accepted June 2, 1990.
Reprint requests: Val Catanzarite, MD, The Perinatal Center, 7930 Frost St., Suite 103, San Diego, CA 92123.
6/1/22872

Table I. Scoring of anatomic visualization

Intracranial anatomy
1. Cranial outline, orbits, midline echo (falx), and cerebellum
2. Ventricles and/or choroid plexus, facial architecture including maxilla and mandible

Spine
1. Ossification centers in normal anatomic relationship in transverse view at each level from occiput to sacrum and in longitudinal view
2. Intact overlaying skin at each level

Heart
1. Four chambers visualized in transverse view; equal atrial and ventricular size
2. Equal wall thickness and intact interventricular septum

Ventral wall
1. Unobstructed longitudinal or transverse view of umbilical insertion
2. Unobstructed longitudinal and transverse views; normal width in both views

Stomach
1. Echolucent area below heart, of appropriate size, in left side of fetal abdomen
2. Echogenic inner wall of stomach seen

Bladder
1. Echolucent area midline, below umbilical insertion, of appropriate size
2. Echogenic inner wall of bladder seen

Umbilical cord
1. Interrupted echoes showing two arteries and vein in longitudinal or transverse view
2. Complete outlines of circular vein and arteries in transverse view or of "spiral rope" appearance in longitudinal view

Genitalia

Male
1. Echodense phallus at an acute angle to the anterior abdominal wall; fetal thighs adducted
2. Distinct outline of fetal penis and/or scrotum

Female
1. Thighs adducted, with clitoral echo seen
2. Distinct outline of "triple-U" configuration of clitoris and labia

tion, cardiac and limb activity, amniotic fluid volume, and placental location. The following measurements were performed: biparietal diameter, head circumference, cerebellum, abdominal circumference, and femur length.

Fetal anatomic structures were scored 0, 1, or 2 according to the visualization criteria of Table I. The sum of these eight scores was used as an overall visualization score (range, 0 to 16). For purposes of data analysis, we described an "adequate" screening examination as one in which the visualization score for each of the seven scores excluding genitalia was at least 1, and an "optimal" screening examination as one in which each of these seven scores was 2.

In addition we recorded time for ultrasonography, maternal height and weight, and measured the abdominal wall thickness from skin to the rectus sheath.

Gestational age was inferred from menstrual dates. If the menstrual dates were uncertain, or differed from ultrasonographic determination by more than 10 days before 22 weeks' gestation, or by more than 14 days at 22 to 26 weeks' gestation, ultrasonographic dating was used.

Table II. Summary of maternal characteristics and selected ultrasonographic observations

	Mean	*SD*	*Range*
Time (min)	17.3	5.6	10-30
Height (in)	64.7	2.6	57-73
Weight (lbs)	154.3	32.3	99-347
Skin to rectus (mm)	19.3	8.3	4-56
Gestational age (wk)	17.9	2.7	13-27
Breech (%)	39.1		
Vertex (%)	43.4		
Anterior placenta (%)	48.0		

Statistical analysis was performed with the STATSOFT 2.1 program (Statsoft, Inc., Tulsa). We used the t test for comparison of independent samples and for determining significance of correlation values, and the F test for significance of multiple regression (R^2) values.

Results

During the study period 589 level II ultrasonographic scans in patients without previously identified anomalies were performed. Leading indications were maternal age (56%), low maternal serum α-fetoprotein (AFP) (20%), elevated maternal serum AFP (13%), and a family history of congenital anomalies (9%).

Sixteen patients were excluded from analysis because of ultrasonographically detected anomalies as follows: neural tube defects (four cases), cystic hygroma (two), isolated two-vessel cord (two), holoprosencephaly and omphalocele (one), holoprosencephaly and oligohydramnios (one), nonimmune hydrops alone (two) or with ventricular septal defect (one), osteogenesis imperfecta (one), multicystic dysplastic kidney with contraleteral renal agenesis (one), and chroid plexus cyst (one).

The remaining 573 patients with normal ultrasonographic findings were the basis for this study. The mean gestational age was 17.9 ± 2.7 weeks; maternal weight ranged from 99 to 347 pounds (mean 154 ± 32 pounds), and the time spent scanning averaged 17 ± 6 minutes. Selected measured parameters are summarized in Table II.

Of the 573 patients, 433 underwent amniocentesis. In four cases trisomy was identified despite normal ultrasonography; three cases of trisomy 21 and one case of trisomy 18 were detected. In no case was a neural tube or abdominal wall defect missed by ultrasonography.

The effects of maternal weight on visualization are summarized in Table III. The overall visualization

score decreased significantly with maternal weight: $r = -0.48$ ($p < 0.0001$). There was a dramatic drop in visualization beyond the 70th percentile in weight: the mean visualization score fell from 11.5 ± 3.0 at <70th percentile (165 pounds) to 9.3 ± 3.7 at the 70th to 90th percentile (165 to 198 pounds) ($p < 0.001$), and to 6.2 ± 3.9 at >90th percentile weight (>198 pounds) ($p < 0.0001$).

Visualization improved with advancing gestational age for the overall score: $r = .34$, $p < 0.001$. Scores for each anatomic feature also improved with gestational age; r values ranged from 0.10 and 0.12 for ventral wall and stomach, respectively, to 0.27 for spine and genitalia and 0.34 for umbilical cord. Each of these was significant at $p < 0.005$. The improvement with gestational age is illustrated for each anatomic feature in Fig. 1.

Visualization of intracranial anatomy by these criteria plateaued at approximately 30% for adequate visualization and 70% for optimal visualization at 19 to 20 weeks' gestation or after. The high rate of inadequate visualization before 15 weeks are largely a result of poor visualization of the cerebellum. After 15 weeks the cerebellum could almost always be seen when the midline structures and orbits were well visualized.

Ossification centers of the spine were visualized in >90% of cases from 15 weeks onward; however, visualization of intact overlying skin continued to improve until 19 to 20 weeks.

All four chambers of the heart could be seen after 16 to 17 weeks in >75% of cases, but demonstration of equal chamber size and wall thickness and an intact septum rose to plateau at 21 to 26 weeks, at only 50%.

The cord insertion could be visualized throughout this gestational age range, but optimal clarity improved with gestational age. There were insufficient numbers for analysis, but there appeared to be a decline in visualization beyond 23 to 24 weeks, because of a relative decline in amniotic fluid volume in relation to fetal size.

The stomach and bladder were both seen in most cases throughout the second trimester; however, for each gestational age group, there was a 5% to 10% rate of nonvisualization of the stomach and a 10% rate for the bladder over the scan duration. A distinct echogenic lining of the stomach was seen in >80% of cases beyond 19 weeks and of the bladder in >90% of cases beyond 19 weeks. Visualization of cord vessels rose throughout the second trimester.

Visualization of the genitalia plateaued at approximately 80% after 20 weeks, and optimal visualization was approximately 60% after 22 weeks.

Multiple regression analysis was performed to determine the effects of measured and inferred parameters on overall visualization. Maternal height, placental position, and fetal lie and presentation did not significantly influence visualization. Maternal weight, skin-to-rectus distance, and gestational age were significant; the combination of weight and gestational age predicted visualization with $R^2 = 0.39$ ($p < 0.0001$, F test). The inclusion of other parameters did not substantially improve the regression fit.

Table III. Visualization by weight percentile

Weight percentile	*Maternal weight (lbs)*	*Visualization score (mean ± SD)*
<10th	<120	12 ± 2.9
10th-20th	120-127	12.6 ± 2.6
20th-30th	127-135	11.6 ± 2.7
30th-40th	135-142	11.9 ± 2.7
40th-50th	142-149	11.3 ± 2.9
50th-60th	149-157	11 ± 2.9
60th-70th	157-165	10.3 ± 3.4
70th-80th	165-177	9.5 ± 3.4
80th-90th	177-198	9.1 ± 3.9
>90th	198-349	6.2 ± 3.9

$p < 0.001$, Weight <70th percentile versus weight 70th to 90th percentile;

$p < 0.001$, Weight 70th to 90th percentile versus weight >90th percentile.

We also analyzed the frequency of "adequate" and "optimal" screening examinations by maternal weight and gestational age. Among patients who weighed <165 pounds (<70th percentile), 13% of patients had "optimal" and 56% had "adequate" screening examinations. Corresponding figures for the 165- to 198-pound group (70th to 90th percentile) were 9% and 34%, and for the >198-pound group (>90th percentile) were 2% and 17%. Breakdown by gestational age is shown in Table IV.

The influence of fetal presentation (vertex vs breech) on visualization of caudal and rostal structures showed a slight but significant improvement in visualization of intracranial anatomy with the fetus in breech presentation (score 1.56 ± 0.60 vs 1.45 ± 0.70, $p = 0.03$); there were no differences in visualization of genitalia or bladder attributable to position.

Comment

We are aware of only one previous study of the rates of visualization of fetal organs in an unselected population undergoing second-trimester ultrasonography. Zador et al.[8] reported results for examinations by ultrasonographers scanning with 3.5 MHz linear and sector transducers. Their criteria for organ visualization differed from ours; however, for each of five organs with comparable criteria—heart, spine, stomach, bladder, and umbilical cord—the rates of visualization reported in our study are higher. These differences may be attributable to ultrasonographer experience or to the use of higher resolution equipment (5 MHz transducers) in our study.

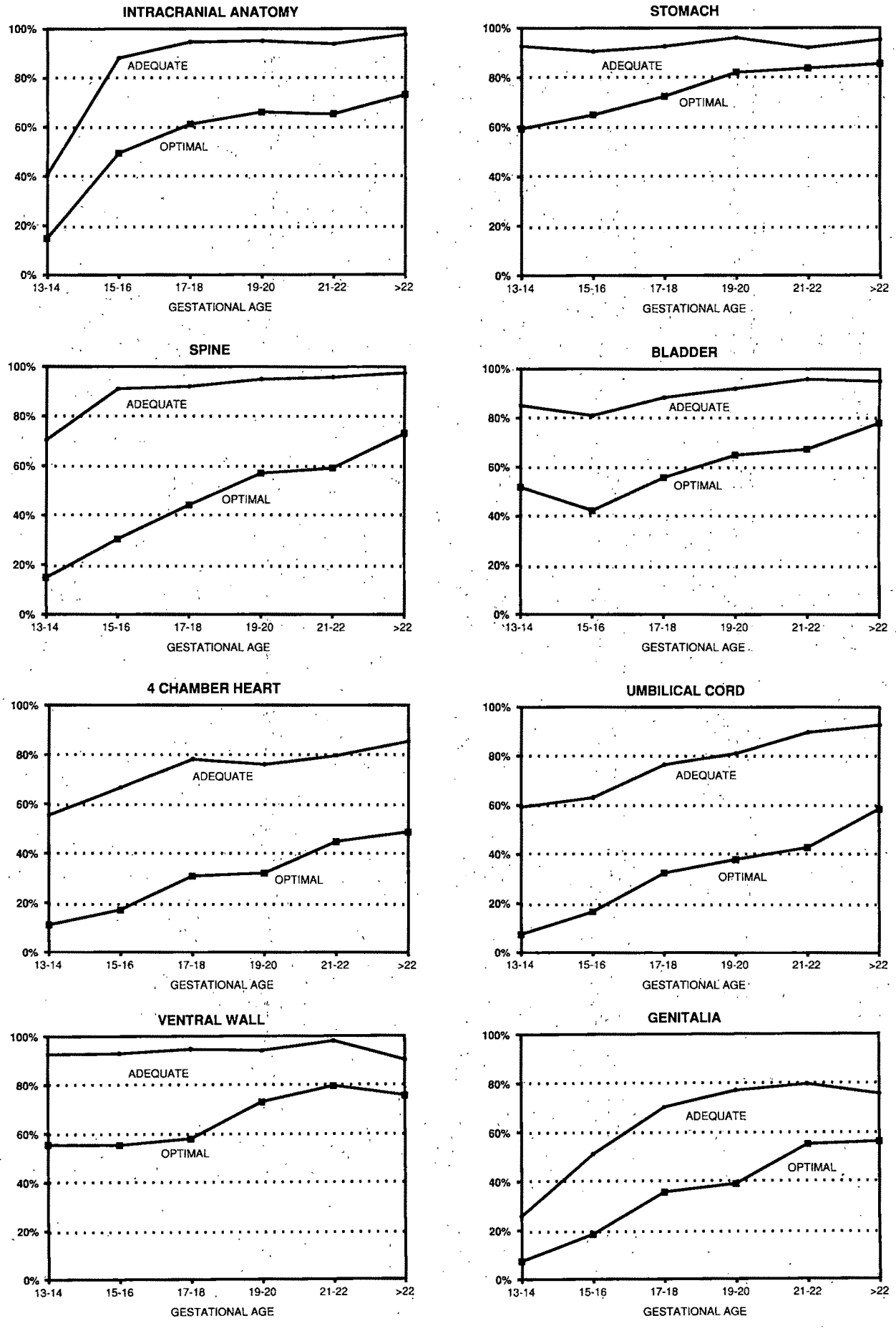


Fig. 1. Anatomic visualization versus gestational age.

Several results of our investigation were unexpected. The rate of "adequate" visualization of the fetal heart was substantially lower than anticipated. With our equipment a view that showed all four chambers was demonstrated in only 80% of patients after 17 weeks' gestation, and visualization adequate as screening for cardiac defects was obtained in <40% of patients at 15 to 20 weeks, and in slightly over half of patients at or beyond 21 weeks. By our criteria "adequate" anatomic screening was accomplished in only 56% of cases overall, and in 221 of 456 (46%) patients at 15 to 20 weeks, when most of our screening scans for congenital anomalies are performed.

The rates of visualization might be improved in any of several ways. The duration of examination would probably increase the visualization rates for at least three points of anatomy—stomach, bladder, and cardiac anatomy. Equipment could be upgraded; the Ultramark-4 with transducers as described is a relatively inexpensive but commonly used machine. Our informal in-house comparisons suggest that image quality is better for several more expensive units (eg, Ultramark-5, Acuson-128). Combined transabdominal and transvaginal scanning would undoubtedly improve visualization rates when anatomy of the head or the fetal rump is obscured by position with the maternal pelvis. Finally, given the same equipment and same time frame, more experienced ultrasonographers might be able to better demonstrate fetal anatomic structures. However, changes in scan duration, ultrasonographic equipment, or ultrasonographic experience are alterations that could not currently be made within the logistic and financial constraints of our ultrasonographic laboratory.

These data have important implications for any ultrasonographic laboratory. The "visualization" scores can be used to compare current ultrasonographic machines with other units. In addition, we now have objective information regarding the frequency of "adequate" and "optimal" screening examinations that may have important medicolegal implications[9] and are of use in counseling patients on the limitations of ultrasonography in our hands.

Finally, our practice has been to perform level II, screening ultrasonograms at 15 to 20 weeks' gestation in the belief that this represented a compromise between optimal fetal anatomic visualization and a time in pregnancy at which termination would still be acceptable to our patients, should an abnormality be found. For patients in the higher weight ranges, and particularly those weighing >90th percentile (198 pounds), the visualization scores and the rates of adequate screening ultrasonography were quite low. Our impression, based on limited numbers of cases at present, is that visualization scores of 8 to 12 are routinely obtained with transvaginal ultrasonography at 12 to 14 weeks. We suspect that transvaginal ultrasonography, at least for the obese patient, will prove to be a superior technique for screening than transabdominal ultrasonography at any time during the second trimester; we plan a prospective comparison to test this hypothesis.

Table IV. Numbers of cases with adequate (score = 1 or 2) and optimal (score = 2) visualization of intracranial, spinal, cardiac, ventral wall, stomach, bladder, and umbilical cord anatomy

Gestational age (wk)	*No.*	*All scores of 1 or 2*	*All scores of 2*
13	5	0 (0%)	0 (0%)
14	22	6 (27%)	0 (0%)
15	61	25 (41%)	5 (8%)
16	107	43 (40%)	6 (6%)
17	119	60 (50%)	11 (9%)
18	69	37 (54%)	6 (9%)
19	55	30 (55%)	8 (15%)
20	45	36 (80%)	3 (7%)
21	24	16 (67%)	6 (25%)
>22	66	41 (62%)	8 (12%)
TOTAL	573	294 (51%)	53 (9%)

REFERENCES

1. American College of Obstetricians and Gynecologists. Ultrasound in pregnancy. Washington DC: American College of Obstetricians and Gynecologists, May 1988, ACOG Technical Bulletin 116.
2. Warsof SL, Cooper DJ, Little D, Campbell S. Routine ultrasound screening for antenatal detection of intrauterine growth retardation. Obstet Gynecol 1986;66:33-9.
3. Green JJ, Hobbins JC. Abdominal ultrasound examination of the first-trimester fetus. AM J OBSTET GYNECOL 1988; 159:165-75.
4. Fleischer AC, Kirchner SG, Thieme GA. Prenatal detection of fetal anomalies with sonography. Pediatr Clin North Am 1985;32:1523-36.
5. Hegge FN, Prescott GH, Watson PT. Sonography at the time of genetic amniocentesis to screen for fetal malformations. Obstet Gynecol 1988;71:522-5.
6. Pilu G, Romero R, Reece EA, Goldstein I, Hobbins JC, Bovicelli L. Subnormal cerebellum in fetuses with spina bifida. AM J OBSTET GYNECOL 1988;158:1052-6.
7. Copel JA, Pilu G, Green J, Hobbins JC, Kleinman CS. Fetal echocardiographic screening for congenital heart disease: the importance of a four-chamber view. AM J OBSTET GYNECOL 1987;157:648-55.
8. Zador IW, Bottoms SF, Tse GM, Brindely BA, Sokol RJ. Nomograms for ultrasound visualization of fetal organs. J Ultrasound Med 1988;7:197-201.
9. Perone N, Carpenter RJ, Robertson JA. Legal liability in the use of ultrasound by office-based obstetricians. AM J OBSTET GYNECOL 1984;150:801-4.

Infertility and eating disorders

Donna E. Stewart, MD, G. Erlick Robinson, MD, David S. Goldbloom, MD, and Charlene Wright, BSc

Toronto, Ontario, Canada

Sixty-six consecutive infertility clinic patients were prospectively screened with the 26-item Eating Attitudes Test and a study questionnaire. Women identified as being at high risk for an eating disorder were then interviewed to confirm or refute the diagnosis. A total of 7.6% of infertility clinic women were found to suffer from anorexia nervosa or bulimia nervosa. If eating disorders not otherwise specified were included, a total of 16.7% of infertility patients were found to suffer from an eating disorder. Among infertile women with amenorrhea or oligomenorrhea 58% had eating disorders. Because women often fail to disclose eating disorders to their gynecologists and may appear to be of normal weight, it is recommended that a nutritional and eating disorder history be taken in infertility patients, particulary those with menstrual abnormalities. It has previously been shown that disordered eating and nutrition can affect menstruation, fertility, maternal weight gain, and fetal well-being. (Am J Obstet Gynecol 1990;163:1196-9.)

Key words: Infertility, eating disorder, anorexia nervosa, bulimia nervosa

Anorexia nervosa, characterized by self-imposed starvation as a result of a relentless pursuit of thinness and a fear of fatness, occurs in a serious form in about 1% of young adult and adolescent women.[1] A related disorder, bulimia nervosa, is defined as episodic patterns of binge eating followed by a variety of methods to counteract the caloric ingestion, including vomiting, starvation, and laxative abuse; bulimia is also accompanied by an intense preoccupation with weight and shape. This may occur in the context of anorexia nervosa or more commonly as a separate syndrome at an actuarially normal body weight. The prevalence of bulimia nervosa as defined by DSM-III-R criteria[2] is 1.7% of adolescent and young adult women.[3] Milder variants of anorexia nervosa and bulimia nervosa that fail to meet criteria for a specific eating disorder, defined by DSM-III-R as eating disorders not otherwise specified, occur in a further 3% to 5% of the female population.[4,5] Eating disorders not otherwise specified is characterized by (1) a person of average weight who does not have binge eating episodes but frequently engages in self-induced vomiting for fear of gaining weight, (2) all of the features of anorexia nervosa in a female subject except absence of menses, or (3) all of the features of bulimia nervosa except the frequency of binge eating episodes.[2] A careful survey of women aged 16 to 35 attending a family practice found a total prevalence of eating disorders and partial syndrome (eating disorders not otherwise specified) of 4%.[5] Eating disorders result in significant morbidity related to episodes of weight loss, vomiting, electrolyte disturbance, gastrointestinal problems, psychiatric sequelae, and reproductive complications.[1] Although it is known that many of these patients, particularly those with anorexia nervosa, have primary or secondary amenorrhea and are infertile,[6] the reproductive aspects of anorexia nervosa and bulimia nervosa have been inadequately studied.

Two early studies examined menstruation, fertility, and pregnancy in patients previously diagnosed as having anorexia nervosa.[7,8] Starkey and Lee[7] found that most anorexic patients reported weight gain, return of menses, and fertility with successful treatment, but women without weight gain did not experience menstrual return or conception. Hart et al.[8] described three women with anorexia nervosa who were induced to ovulate with human menopausal gonadotropin–human chorionic gonadotropin but then experienced significant fetal loss because of miscarriages, multiple pregnancies, premature births, and low birth weights.

Investigators have also previously found that some women with amenorrhea or infertility who are seen by gynecologists actually have undisclosed eating disorders.[9-13] An early study by Fries[13] found a high proportion of women with an eating disorder among 30 women with secondary amenorrhea associated with weight loss. Nillius[11] reported that 34% of amenorrheic women in his study were found to have amenorrhea caused by self-induced weight loss. The diagnosis of

From the Departments of Psychiatry and Obstetrics and Gynecology, St. Michael's Hospital and Toronto General Hospital, University of Toronto.

This work was partially supported by a Canadian Psychiatric Research Foundation grant to Drs. Stewart and Robinson.

Received for publication October 17, 1989; revised April 25, 1990; accepted May 18, 1990.

Reprint requests: Dr. Donna Stewart, St. Michael's Hospital, 30 Bond St., Toronto, Ontario, Canada M5B 1W8.

6/1/22461

such underlying problems may be hampered by the fact that some bulimic women appear to be of normal weight by actuarial or population mean standards. However, longitudinal weight history may reveal premorbid obesity that led to highly restrictive dieting coupled with bulimic behavior.[14] A recent Australian study using the Eating Disorders Inventory also found elevated drive for thinness and interoceptive awareness subscales to point toward disordered eating in 30 infertility clinic patients,[15] but these investigators did not conduct confirmatory interviews for eating disorders. Bates et al.[12] found that 47 women with unexplained infertility and menstrual dysfunction were practicing weight control by caloric restriction to maintain a fashionable body habitus. The report did not state whether formal eating disorders were found in any woman in this study. When 36 of these women followed a dietary regimen designed to increase their weight to predicted ideal body weight, menstruation resumed in most, with subsequent spontaneous conception. It is of interest that 97% of these infertile women and their husbands had been evaluated previously for infertility with a variety of diagnostic gynecologic studies without weight loss being identified as the cause of the infertility.

The aim of the present study was to ascertain prospectively the prevalence of eating disorders (anorexia nervosa, bulimia nervosa, and eating disorders not otherwise specified) in a group of women with infertility attending a university reproductive biology service.

Material and methods

Consecutive women with infertility seen at a university reproductive biology service were assessed by means of a standardized, reliable, valid self-report measure, the 26-item Eating Attitudes Test (EAT-26), which is presently in widespread use as a screening instrument for eating disorders.[16] The EAT-26 focuses on specific dysfunctional beliefs and behaviors associated with anorexia nervosa and bulimia nervosa.[16] The women also completed a study questionnaire on current and past weights, menstrual history, and gynecologic history. All women identified on screening tests as having possible eating disorders (EAT-26 score ≥ 20) or who reported a past or current eating disorder were interviewed by one of the investigators (D. E. S.) to confirm or refute the diagnosis of an eating disorder by DSM-III-R criteria. Statistical analyses were performed by one-way analysis of variance.

Results

Sixty-six women completed the EAT-26 and the study questionnaire. There were three refusals to complete these questionnaires, resulting in a 96% response rate. One woman who refused later acknowledged to infertility clinic staff that she had been treated in the past for bulimia nervosa and was still regularly binge eating and inducing vomiting. The three women who refused to complete the questionnaires are not included in the data.

The mean age of the women completing the questionnaires was 30.4 years (range 21 to 39). Their mean weight was 103% ideal body weight (range 72% to 143%). Ideal body weight was defined as the average weight for a given height and age, determined from statistics compiled in 1979 by the Association of Life Insurance Medical Directors of America and Society of Actuaries. Twelve women had abnormal menses (amenorrhea or oligomenorrhea).

According to the cutoff score described above for the EAT-26, 12 women were identified as possible cases of eating disorders. In addition, two women who did not meet the criteria disclosed on the study questionnaire that they were being treated for an eating disorder and one woman who scored zero on all EAT-26 items was identified by a note on her questionnaire saying "I am *not* bulimic" and an allegation of bulimia made by her sister. Clinical interviews with all 15 women confirmed that 11 had an eating disorder. Utilizing DSM-III-R criteria, four women had bulimia nervosa, one had anorexia nervosa, and six had eating disorders not otherwise specified. This results in a prevalence of a formal specific eating disorder (anorexia nervosa or bulimia nervosa) of 7.6% (16.7% when eating disorders not otherwise specified are included). The four women identified as possible cases on screening but not confirmed by interview were all overweight (ideal body weight = 120%), dieting, and preoccupied by food. Of the 12 women in the total sample with abnormal menses, seven (58.3%) suffered from anorexia nervosa, bulimia, or eating disorders not otherwise specified (Table I).

There was no significant difference in the percent ideal body weight between women with eating disorders and women without eating disorders. Analysis of variance was used to compare scores of amenorrheic and oligomenorrheic women with those of women with normal menses. Total scores on EAT-26 were significantly higher ($p < 0.0001$) in the abnormal menses group than in the normal menses group. Although all four amenorrheic women had significantly lower percent ideal body weight than all other women ($p = 0.04$), the percent ideal body weight was not significantly different between normal and the total abnormal menses group.

Comment

We found a 7.6% prevalence by DSM-III-R criteria of a specific eating disorder (anorexia nervosa or bulimia nervosa) in consecutive women seen in a university hospital clinic for infertility. This rate is two to four

Table I. Menses and eating disorder in 66 infertility clinic women

Eating disorder	*Menses*			*Total*
	Normal	*Oligomenorrhea*	*Amenorrhea*	
Anorexia nervosa	0	0	1	1
Bulimia nervosa	0	2	2	4
Eating disorder not otherwise specified	4	2	0	6
No eating disorder	50	4	1	55
TOTAL	54	8	4	66

times that predicted by population and general practice studies of women in this age group.[1, 3, 5] The 12 women with menstrual abnormalities in our sample had a higher prevalence (58%) of eating disorders than normally cycling women (7%), suggesting that infertile women with menstrual abnormalities are at even higher risk of suffering from an eating disorder. Other investigators have described the endocrine findings associated with menstrual cycle abnormalities in women with anorexia nervosa and bulimia nervosa. In general, these women tend to have fewer luteining hormone secretory spikes and a trend toward lower mean 24-hour luteinizing hormone levels than controls.[16] Stimulation with gonadotropin-releasing hormone produced elevated luteinizing hormone responses in the bulimic group and blunted luteinizing hormone responses in the anorexic group.[16] Estradiol levels were uniformly lower in women with anorexia nervosa,[17] and stimulation with estradiol revealed diminished luteinizing augmentative responses and a trend toward diminished follicle-stimulating hormone augmentative responses in patients with anorexia nervosa and bulimia nervosa as compared with those of controls.[16] Other studies have shown that approximately 50% of normal weight women with bulimia nervosa have menstrual abnormalities with impaired follicular maturation caused by impaired gonadotropin secretion.[17, 18] Moreover, it has been shown in normal young women of normal body weight who diet for 6 weeks (800 to 1000 kcal/day) that a variety of changes in endocrinologic function develop with actual disruption of menstrual cycles for 3 to 6 months after dieting in approximately 20%.[19] Recently investigators have shown that women with eating disorders not otherwise specified also frequently have menstrual dysfunction.[21] If women with eating disorders not otherwise specified are included in our prevalence figures for an eating disorder in infertility clinic patients, the rate rises to 16.7%, a finding of concern that warrants further study. Eating disorders may play an important etiologic role in infertility because recent work has shown that 73% of normal infertile patients who were below ideal body weight because of caloric restriction conceived spontaneously when their weight was corrected.[12]

None of the women we identified with eating disorders had divulged this problem to the infertility clinic gynecologist before the introduction of the screening questionnaires. The exposure to these questionnaires appears to have encouraged more open disclosure about eating disorders. Five women wrote notes on their questionnaires stating they were worried that abnormal eating habits had caused their infertility. On psychiatric interview all four women with bulimia nervosa and three of six women with eating disorders not otherwise specified confided that they had previously sought treatment for an eating disorder but failed to report it to the gynecologist. All expressed a concern that they had "damaged" themselves by their disordered eating behaviors but expressed relief at now being able to discuss this further.

Patients often fail to volunteer information about eating disorders to their gynecologists and may sometimes appear to be of normal weight (in fact, percent ideal body weight was a poor predictor of an eating disorder in this and other studies)[5]; therefore, when infertile patients are assessed, especially those with menstrual abnormalities, there is a need for routine careful inquiry into nutritional intake and eating disorder history. Relevant clinical screening inquiries include a history of highest and lowest ever weights, desired body weight, average daily caloric intake, dieting, and alternative attempts at weight control. For routine evaluation, "consciousness raising," or research purposes, screening of infertility clinic patients with the EAT-26 using a cutoff score of ≥ 20 will identify a group of women at high risk for an eating disorder. Given the brevity, ease of scoring, and ability of the EAT-26 to correctly identify 73% of patients with eating disorders in our study, it appears to be a fairly useful screening instrument in this population. Women identified as high risk on screening can then be referred for further assessment to confirm or refute the diagnosis of an eating disorder. Women with false-positive results on the EAT-26 were characteristically overweight dieters, a finding confirmed by other investigators.[22] It should be borne in mind that the EAT-26 will not identify all women with eating disorders, especially those who deny symptoms. However, an awareness of the frequency of

eating disorders in infertility patients followed by careful clinical inquiry should correctly identify most cases.

All women in this study who had a confirmed eating disorder were advised to have psychiatric treatment and nutritional counseling. In clinical practice, women found to have an eating disorder should be treated for this before infertility investigations and treatments are pursued. We have previously shown that women whose eating disorders persist during pregnancy gain less weight, have more complications of pregnancy, have smaller babies with lower 5-minute Apgar scores, experience more difficulties in postpartum adjustment, and have more problems with breast-feeding.[10] It is not known if the high rate of spontaneous conception with correction in weight is applicable to formal eating disorders, but our earlier work indicates that successful treatment of eating disorders reduces morbidity of the pregnant woman and her offspring.[10]

We thank Dr. C. Redmond, Dr. C. Derzko, and Dr. R. Casper for encouraging their infertility patients to participate in this survey, and Dr. M. Morris for contacting the high-risk women identified by the screening instruments.

REFERENCES

1. Garfinkel PE, Garner D. Anorexia nervosa: a multidimensional perspective. New York: Bruner/Mazel, 1982: 101, 307-26.
2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 3rd ed revised. Washington DC: American Psychiatric Associaton, 1987.
3. Ben-Tovim DI. DSM-III, draft DSM-III-R and the diagnosis and prevalence of bulimia in Australia. Am J Psychiatry 1988;145:1000-2.
4. Button EJ, Whitehouse A. Subclinical anorexia nervosa. Psychol Med 1981;11;509-16.
5. King MB. Eating disorders in general practice. Br Med J 1986;293:1412-4.
6. Reid RL, VanVugt DA. Weight-related changes in reproductive function. Fertil Steril 1987;48:905-13.
7. Starkey TA, Lee RA. Menstruation and fertility in anorexia nervosa. Am J Obstet Gynecol 1969;105:374-9.
8. Hart T, Kase W, Kimball CP. Introduction of ovulation and pregnancy in patients with anorexia nervosa. Am J Obstet Gynecol 1970;108:580-4.
9. Stewart DE, MacDonald OL. Hyperemesis gravidarium and eating disorders in pregnancy. In: Abraham S, Llewellyn-Jones D, eds. Eating disorders and disordered eating. Sydney: Ashwood House, 1987:52-5.
10. Stewart DE, Raskin J, Garfinkel PE, MacDonald OL, Robinson GE. Anorexia nervosa, bulimia, and pregnancy. Am J Obstet Gynecol 1987;157:1194-8.
11. Nillius SJ. Psycho-pathology of weight-related amenorrhea. In: Jacobs, HS, ed. Advances in gynaecological endocrinology. London: Royal College of Obstetricians and Gynaecologists, 1978:118-30.
12. Bates GW, Bates SR, Whitworth NS. Reproductive failure in women who practice weight control. Fertil Steril 1982;37:373-8.
13. Fries H. Secondary amenorrhea, self-induced weight reduction and anorexia nervosa. Acta Psychiatr Scand 1974;248(suppl):1-69.
14. Garfinkel PE, Moldofsky H, Garner DM. The heterogeneity of anorexia nervosa: bulimia as a distinct group. Arch Gen Psychiatry 1980;37:1036-40.
15. Allison S, Kalucy R, Gilchrist P, Jones W. Weight preoccupation among infertile women. Int J Eating Dis 1988;7:743-8.
16. Garner DM, Olmsted MP, Bohr Y, Garfinkel PE. The Eating Attitudes Test: psychometric features and clinical correlates. Psychol Med 1982;12:871-8.
17. Devlin MJ, Walsh BT, Katz JL, et al. Hypothalmic-pituitary-gonadal function in anorexia nervosa and bulimia. Psychiatry Res 1980;28:11-24.
18. Copeland PM, Herzog DB. Menstrual abnormalities. In: Hudson JI, Pipe HG, eds. The psychobiology of bulimia. Washington DC: American Psychiatric Press, 1987:31-54.
19. Pirke KM, Dogs M, Fichter MM. Tuschil RJ. Gonadotrophins, oestradiol, and progesterone during the menstrual cycle in bulimia nervosa. Clin Endocrinol 1988;29:265-70.
20. Pirke KM, Ulrich S, Lemmel W, Krieg JC, Berger M. The influence of dieting on the menstrual cycle of healthy young women. J Clin Endocrinol Metab 1985;60:1174-9.
21. Kreipe RE, Strauss J, Hodgman CH, Ryan RM. Menstrual cycle abnormalities and subclinical eating disorders: a preliminary report. Psychosom Med 1989;51:81-6.
22. Wells JE, Coope PA, Gabb DC, Pears RK. The factor structure of the Eating Attitudes Test with adolescent school girls. Psychol Med 1985;15:141-6.

Postpartum osteomyelitis caused by group B streptococcus

K. Berkowitz, MD, and R. McCaffrey, MD
New York, New York

Postpartum infections caused by group B streptococci are generally limited in scope. We report a case of vaginal colonization with group B streptococcus that progressed in the postpartum period to osteomyelitis that necessitated total hip replacement. The patient had no risk factors predisposing to streptococcal osteomyelitis. An altered immune status in pregnancy and intrapartum bacteremia may be involved in the pathogenesis of this infection. (Am J Obstet Gynecol 1990;163:1200-1.)

Key words: Colonization, osteomyelitis, group B streptococcus

Postpartum infections associated with vaginal group B streptococcal colonization are commonly limited to adjacent anatomic structures such as the uterus and bladder. Transmission of the organism to the newborn has been associated with a variety of neonatal complications including sepsis and osteomyelitis.[1] We report an unusual complication of group B streptococcal infection in a woman in whom severe postpartum osteomyelitis developed and required hip replacement.

Case report

A 34-year-old nulligravid woman was seen at term in active labor. Her medical history was unremarkable. She had conceived with clomiphene therapy. The antepartum course had been uneventful. The membranes ruptured spontaneously 24 hours before delivery without any signs of chorioamnionitis. The vaginal delivery was uncomplicated and a partial third-degree episiotomy was repaired easily. A routine intrapartum culture for group B streptococcus was noted to be positive after delivery. The patient had no evidence of infection and was not treated. The infant had no signs of streptococcal disease. The patient complained of pain at the episiotomy site on the third postpartum day. The site was clean, she was afebrile, and her discomfort resolved with analgesia. She remained afebrile on the fourth postpartum day but complained of pain in the left hip and buttock. Physical examination revealed no limitation of motion in the left hip. There were no signs of deep vein thrombosis. Hip radiographs were normal. Her symptoms resolved with analgesia. She was discharged on the fifth postpartum day.

At home the patient experienced increasing left hip pain radiating to the left buttock and knee that was not relieved by analgesics. She was unable to raise her left leg by the seventh postpartum day and was readmitted for evaluation. Neurologic examination revealed no motor or sensory deficits. Venogram of the lower extremities and tomographic scans of the lumbar-sacral spine and pelvis were unremarkable. Pelvic ultrasonogram showed no evidence of hematoma. The patient became febrile to 39.0° C with an associated elevation in white blood cell count to 20,000 cells/mm^3 and sedimentation rate of 145. A three-phase bone scan revealed synovitis of the left hip. Hip joint aspiration yielded clear sterile fluid. Empiric antibiotic therapy with cefazolin and tobramycin was initiated on the fourteenth postpartum day when hip radiographs showed narrowing of the left hip joint and demineralization of the acetabulum. A second hip joint aspirate grew group B streptococcus on the fifteenth postpartum day. A synovial biopsy showed purulent granulation tissue and grew group B streptococcus. Cultures were negative for mycotic disease or acid-fast bacilli. Rheumatoid factor and antinuclear antibody determinations were negative. The patient improved after initiation of high-dose parenteral penicillin G and minidose heparin therapy. She was discharged on the thirtieth postpartum day to begin rehabilitative therapy. Serial hip radiographs showed progressive deterioration of the hip joint. A bone scan performed 6 months postpartum revealed avascular necrosis of the left acetabulum. She then underwent low-friction arthroplastic total hip replacement with a Charnley prosthesis.

Comment

Septic arthritis and osteomyelitis caused by group B streptococcal infection is seen infrequently in neonates and children. It is very rare in adults but has been reported in patients with diabetes mellitus or altered immune status.[2] Colonization reservoirs and opsonic antibody-complement abnormalities may be involved in the pathogenesis of this infection.[2] Postpartum osteomyelitis caused by group B streptococcus has not previously been reported. The potential for group B streptococcal colonization to progress to a disseminated infection such as postpartum osteomyelitis is documented

From the Department of Obstetrics and Gynecology, Columbia Presbyterian Medical Center, Sloane Hospital for Women.
Received for publication March 26, 1990; accepted April 4, 1990.
Reprint requests: K. Berkowitz, MD, Department of Obstetrics and Gynecology, Columbia Presbyterian Medical Center, Sloane Hospital for Women, 622 W. 168th St., New York, NY 10032.
6/1/21412

in this case report. It can be postulated that hematogenous spread of the organism to the hip was facilitated by bacteremia accompanying the process of labor and delivery. The patient's altered immune status of pregnancy may have increased her susceptibility to the development of infection.

Disseminated postpartum infections require a concerted diagnostic effort aimed at identifying the reservoir responsible for bacterial seeding. Organisms found in antenatal or intrapartum vaginal cultures are likely sources of infection. Information gained from vaginal cultures may help the clinician to initiate definitive rather than empiric therapy in women with unclear causes for severe postpartum infections.

REFERENCES

1. Baxter MP, Finnegan MA. Skeletal infection by group B β-haemolytic streptococci in neonates. J Bone Joint Surg 1988(B);70:812-4.
2. Stevens DL, Haburchak DR, McNitt TR, Everett ED. Group B streptococcal osteomyelitis in adults. South Med J 1978;71:1450-1.

Antenatal spontaneous perforation of the extrahepatic biliary tree

Syam Chilukuri, MD, Virgilio Bonet, MD, and Mason Cobb, MD
East Lansing, Michigan

Spontaneous perforation of the biliary system is an unusual neonatal phenomenon that is rarely recognized at birth. To date, it has not been reported antenatally. A 16-year-old pregnant adolescent had an ultrasonogram at 25 weeks of pregnancy that revealed ascites in the fetus. After the infant was delivered at 32 weeks, a hepato-iminodiacetic acid scan showed a spontaneous rupture of the common bile duct. The infant was treated with external drainage of the biliary tree and recovered well. This case demonstrates that spontaneous perforations of the bile ducts can occur much earlier than the usually described 2 to 12 weeks after birth, can be diagnosed antenatally, and should be added to the list of causes of fetal ascites. (Am J Obstet Gynecol 1990;163:1201-2.)

Key words: Fetal ascites, spontaneous perforation of bile duct

Spontaneous perforation of the common bile duct is uncommon in neonates but has been well documented as the second most common surgical cause of jaundice in the newborn.[1] However, it has not been reported to have occurred in utero, with most cases occurring between 2 and 12 weeks after birth.[1] This case illustrates fetal ascites diagnosed before birth, which subsequently was shown to be antenatal perforation of the common bile duct.

Case report

A 16-year-old white adolescent (gravida 1, para 0, abortions 0) was progressing normally until approximately 25 weeks of gestation when the fetus was found to have developed ascites on a routine ultrasonogram. The mother was not found to have polyhydramnios but preeclampsia subsequently developed and the infant girl was delivered via cesarean section at 32 weeks' gestation.

The infant was delivered without incident; Apgar scores were 7 and 9, birth weight was 1940 gm, and head circumference was 30 cm. The infant was thus normal size for 32 weeks of gestation. She was not jaundiced. The abdomen was distended but there was no organomegaly. An ultrasonogram revealed no abnormality except the ascites.

Liver enzyme levels were normal. A hepato-iminodiacetic acid scan was obtained and extravasation of the radionuclide from the extrahepatic biliary tree established the diagnosis of a spontaneously perforated duct.

The infant's abdomen was explored 2 days after birth when she was found to have 150 to 200 ml of free bile in the peritoneal cavity. An operative cholecystogram showed flow into the duodenum but no extravasation. External drainage of the biliary tree was performed via a cholecystostomy. The postoperative course was un-

From the Department of Surgery, Michigan State University.
Received for publication February 13, 1990; accepted May 1, 1990.
Reprint requests: Dr. Virgilio Bonet, Department of Surgery, Michigan State University, East Lansing, MI 48824.
6/1/22088

eventful except for a single reaccumulation of ascites that was tapped and was found to be chylous. A repeat hepato-iminodiacetic acid scan before discharge was normal. The infant remains healthy and is thriving at 18 months' follow-up.

Comment

Spontaneous perforation of the extrahepatic biliary ducts should be added to the list of causes of fetal ascites. Among the known causes of fetal ascites are urinary tract abnormalities, gastrointestinal diseases, biliary atresia, storage diseases, malignancies, Rh or ABO incompatibility leading to isoimmunization, and other causes of fetal hydrops.[1,2]

Although the cause is still not known, the location of the spontaneous perforation is usually at the junction of the cystic duct and the common hepatic duct.[1] This finding suggests a tendency for developmental errors at this site. The theory advanced by Lilly et al.[1] is that the perforation is a result of a localized embryonic mural malformation in this area. The same area is also the most frequent site of choledochal cyst, which further supports this thesis.

The common period of discovery is between 4 and 12 weeks of age, although it is occasionally seen at birth. The pathognomonic finding is biliary ascites, but a definitive preoperative diagnosis can be made by an intravenous hepatocholangiogram with technetium 99m-Tc disofenin or other isotopes of technetium 99m-Tc, such as hepato-iminodiacetic acid.

Treatment is surgical and can be accomplished with external drainage.[1] A cholecystostomy may be added as in this case. Other internal drainage procedures have also been advocated by some authors but are not well accepted.

Antenatal diagnosis has advanced clinical acumen in recognizing and treating many congenital surgical disorders. To date, antenatal spontaneous perforation of the extrahepatic bile ducts has not been reported.[1,2]

This case also reveals that bile duct perforation can occur at least as early as the twenty-fifth gestational week. Previously this was thought to occur after birth, although the defect itself is congenital.[1]

Extrahepatic bile duct perforation should be considered in the differential diagnosis of fetal ascites. A surgical consultation with the mother and family and plans for postnatal surgical care are indicated in such cases.

REFERENCES

1. Lilly J, Weintraub W, Altman R. Spontaneous perforation of the extrahepatic bile ducts and bile peritonitis in infancy. Surgery 1974;75:664-73.
2. Mir N, Perlman M. Non-immune fetal ascites: an appraisal of etiology and outcome—a clinical and pathology review of 35 cases. Asia Oceania J Obstet Gynaecol 1987;13:287-92.

Antenatal diagnosis of molybdenum cofactor deficiency

R. George F. Gray, PhD,[a] Anne Green, MSc,[a] Shishta Nadda Basu, MRCOG,[b] Glyn Constantine, MD,[b] Roy G. Condie, MD,[b] Claude Dorche, PhD,[c] Christine Vianey-Liaud, PhD,[c] and Pierre Desjacques, PhD[c]

Birmingham, United Kingdom, and Lyon, France

Analysis of uncultured chorionic villus material from a woman at risk of fetus with sulfite oxidase deficiency revealed a deficiency of sulfite oxidase. This was confirmed on termination of the pregnancy. (AM J OBSTET GYNECOL 1990;163:1203-4.)

Key words: Molybdenum cofactor deficiency, chorionic villus sampling, amniocentesis

Molybdenum cofactor deficiency is a rare autosomal recessive disorder that, in the neonate, appears with fits, choreiform movements, and lens dislocation. Biochemically it is characterized by a deficiency of sulfite and xanthine oxidases and the accumulation in the body fluids of sulfite, thiosulfate, S-sulfocysteine, xanthine, and hypoxanthine. We report a further case with details of a successful antenatal diagnosis by chorionic villus sampling and amniocentesis.

Case history

The index case was the fourth pregnancy of a Pakistani Moslem couple who were first cousins. There had been delivery of a healthy male, a female who died of pneumonia at age 1 month, and a spontaneous miscarriage. The pregnancy was uneventful and a female was born (birth weight, 3.75 kg) at term. Apgar scores at birth were 9 and 10 at 1 and 5 minutes, respectively. However, the infant ate poorly and underwent an apnea attack at 10 hours, with generalized convulsions and opisthotonos. An electroencephalogram revealed epileptogenic abnormalities, suggesting a progressive encephalopathy. A computerized axial tomography scan of the brain revealed areas of low attenuation with slight ventricular dilation. Intermittent convulsions continued, ventricular tachycardia developed and she died on day 12 after birth. Plasma uric acid was substantially reduced (<1 mmol/L), whereas urinary xanthine (433 mmol/mmol creatinine) and hypoxanthine (10 mmol/mmol creatinine) were raised. Unfortunately insufficient samples were available for the measurement of sulfur-containing metabolites and cultured fibroblasts were not available. The clinical picture and the abnormal purine metabolites led us to the presumptive diagnosis of molybdenum cofactor deficiency.

From the Department of Clinical Chemistry, The Children's Hospital,[a] Department of Obstetrics and Gynaecology, Dudley Road Hospital,[b] and Laboratoire de Biochimie, Hôpital Debrousse.[c]
Received for publication March 13, 1990; accepted March 27, 1990.
Reprints not available.
6/1/21109

Antenatal diagnosis

The mother was first seen with her fifth pregnancy at 6 weeks' gestation. A chorionic villus sample was taken transcervically at 11 weeks' gestation. The uncultured chorionic villus tissue revealed a total deficiency of sulfite oxidase, whereas hexosaminidase activity was present. However, because of transportation problems between the laboratories in Birmingham and Lyon, the specimen was in transit for 3 days. Inasmuch as it was not known how stable the enzyme was under these condition, an amniocentesis was carried out at 16 weeks' gestation. The amniotic fluid supernatant revealed increased concentrations of S-sulfocysteine (22 μmol/L, normal values (<1) and a low concentration of cysteine (15 μmol/L, 2 SD, normal range 72 to 134). The amniotic fluid supernatant uric acid concentration was normal (165 μmol/L, normal range 95 to 202). An extraamniotic termination of pregnancy was carried out at 17 weeks' gestation. The cultured amniotic fluid cells and abortus fibroblasts revealed a gross deficiency of sulfite oxidase activity, whereas the abortus liver revealed no detectable sulfite and xanthine oxidase activity. A postmortem examination revealed no histologic abnormalities in any tissues on light or electron microscopy.

Comment

The assay of sulfite oxidase in uncultured chorionic villus material permits a diagnosis of molybdenum cofactor deficiency within 7 days of sampling, whereas analysis of purine and sulfur-containing metabolites in the amniotic fluid gives a more rapid result than the

enzyme assay on cultured amniotic fluid cells. Amniotic fluid uric acid concentrations are clearly not useful diagnostically.

We thank Bill Mackenzie of Marston Green Hospital, Birmingham, England, for carrying out the chorionic villus sampling, Dr. Anne Aukett of Dudley Road Hospital for her help with the index case, and Kevin Carpenter of the Sheffield Children's Hospital for the purine metabolite assays.

REFERENCE

1. Brown GK, Scholem RD, Croll HB, Wraith JE, McGill JJ. Sulfite oxidase deficiency: clinical, neuroradiologic and biochemical features in two new patients. Neurology 1989; 39:252-7.

Preoperative evaluation of serum CA 125, TAG 72, and CA 15-3 in patients with endometrial carcinoma

John T. Soper, MD,[a] Andrew Berchuck, MD,[a] George J. Olt, MD,[a] Andrew P. Soisson, MD,[a] Daniel L. Clarke-Pearson, MD,[a] and Robert C. Bast, Jr., MD[b]

Durham, North Carolina

We evaluated 109 women with endometrial carcinoma to determine the accuracy of preoperative tumor-associated antigen levels (CA 125, CA 72, CA 15-3) for prediction of extrauterine disease and whether TAG 72, CA 15-3, or both would improve the predictive value of CA 125 alone. Eleven (12%) of 80 patients with disease confined to the uterus or positive cytologic findings had CA 125 values >35 U/ml versus 12 (65%) of 20 patients with extrauterine metastasis. Therefore CA 125 values had sensitivity of 65% and specificity of 88%. The TAG 72 level was elevated (>6 U/ml) in 4% of patients with localized disease and 30% with metastasis. CA 15-3 was elevated (> 30 U/ml) in 17% and 65% in these categories, respectively. TAG 72 or CA 15-3 levels did not improve the combination of sensitivity and specificity of CA 125 alone. In addition, only one of 10 patients with microscopic metastasis (three cases) or positive peritoneal cytology (seven) had elevation of any of these tumor-associated antigen levels. Failure to detect occult metastasis and a high false-positive rate limit the role of these tumor-associated antigen assays in the preoperative evaluation of patients with endometrial carcinoma. (Am J Obstet Gynecol 1990;163:1204-9.)

Key words: Endometrial cancer, tumor-associated antigens, CA 125, TAG 72, CA 15-3

It has been established that women with early clinical stage endometrial carcinoma frequently have extrauterine metastasis detected only through staging laparotomy. The International Federation of Gynecology and Obstetrics (FIGO) recently designated a surgical staging system for endometrial cancer.[1] Although the risk of extrauterine metastasis can be predicted on the basis of several pathologic features,[2] only histologic grade and depth of the uterine cavity are known preoperatively in the majority of women with endometrial cancer. The development of a sensitive and specific noninvasive test that could be applied preoperatively to predict the presence of extrauterine metastasis would allow selection of patients at high risk for extrauterine disease who would most likely benefit from comprehensive surgical staging procedures, including selective pelvic and paraaortic lymphadenectomy.

Niloff et al.[3] first reported the association of elevated CA 125 levels with advanced and recurrent endometrial carcinomas. Recent reports by Duk et al.[4] and Patsner et al.[5] suggested that preoperative elevations of serum CA 125 levels might identify women with clinically early endometrial carcinomas who have occult metastatic disease. Berchuck et al.[6] subsequently reported that increasing expression of CA 125 in the primary endo-

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology,[a] and the Comprehensive Cancer Center,[b] Duke University Medical Center.
Supported in part by grant 5R01 CA39930 and Gaston County Cancer Society Clinical Research Fund.
Received for publication March 1, 1990; revised May 29, 1990; accepted June 8, 1990.
Reprint requests: John T. Soper, MD, P.O. Box 3079, Duke University Medical Center, Durham, NC 27710.
6/1/23001

metrial cancer was associated with increased metastatic potential. On the basis of these reports, we conducted a prospective evaluation of preoperative sera from women with endometrial carcinomas to determine the value of CA 125 elevations in predicting extrauterine metastasis. Levels of two other tumor-associated antigens, TAG 72 and CA 15-3 were also measured to determine whether levels of these tumor-associated antigens in combination with CA 125 would improve the sensitivity or specificity achieved with CA 125 alone.

Material and methods

One hundred nine women with endometrial adenocarcinomas treated between 1986 and 1989 had pretreatment sera available in sufficient quantity to determine levels of CA 125, TAG 72, and CA 15-3. Sera were obtained within 1 week before primary operation or institution of definitive therapy in one patient with clinically advanced disease. All patients underwent preoperative evaluation consisting of physical examination, pelvic examination with the patient under anesthesia, chest x-ray film, and other appropriate radiographic studies. Surgical stage was assigned according to FIGO criteria[1] based on the results of surgicopathologic findings at laparotomy.

In patients with disease grossly confined to the uterus at laparotomy, bilateral selective pelvic and paraaortic lymphadenectomy was performed unless the patient had a grade 1 or 2 lesion confined to the endometrium or invading the inner myometrium by frozen section of the hysterectomy specimen. Peritoneal washings for cytologic testing and omental biopsy were performed in all patients. One woman with clinical evidence of systemic and vaginal metastasis was treated without laparotomy. Ten patients with gross intraperitoneal carcinomatosis underwent hysterectomy, bilateral salpingo-oophorectomy, and tumor debulking without lymph node sampling.

Preoperative sera were separated, frozen, and stored at −70° C. Tumor-associated antigen assays were performed after completion of initial operation and clinicians were blinded to the results of the assays.

CA 125 assay. An immunoradiometric assay (Centocor, Malvern, Pa.) was performed as previously described[7, 8] to quantitate CA 125 levels in 100 μl portions of serum. All assays were performed in duplicate and were compared with a normal curve constructed by use of control samples with known quantities of CA 125 antigen. A value of 35 U/ml was selected as threshold for this study.[7, 8]

Tumor-associated glycoprotein (TAG 72) assay. A solid-phase double-determinant radioimmunometric assay (Centocor) was also used to quantitate serum TAG 72 levels. All assays were performed in duplicate. Polystyrene beads coated with monoclonal antibody B72.3 were incubated simultaneously with 100 μl serum samples combined with 100 μl iodine 125–labeled B72.3.[9-11] Specimens were incubated for 18 hours in a 4° C water bath. The polystyrene beads were washed four times with 5 ml distilled water and radioactivity was determined by a gamma scintillation counter. Standard curves were constructed with known quantities of TAG 72 from a standard source. A level of 6 U/ml was assigned as a threshold for this study.

Serum CA 15-3 assay. The serum CA 15-3 assay was also a solid-phase double-determinant immunoradiometric assay (Centocor). Polystyrene beads coated with monoclonal antibody 115.D8[12, 13] were incubated with 200 μl of serum for 60 minutes at room temperature. Each well was rinsed three times with a total of 15 ml distilled water. Two hundred microliters of mouse monoclonal antibody DF3 labeled with ^{125}I was added for an additional 60 minutes. The beads were rinsed with 15 ml distilled water and radioactivity was counted in a γ-scintillation counter. A standard curve was obtained with samples that contained known concentrations of CA 15-3. Unknown sera were run in duplicate and the corresponding concentration of CA 15-3 was determined directly from the standard curve. A threshold level of 30 U/ml was assigned for this study.

The χ^2 test was used for analysis of trends with significance declared at the $p < 0.05$ level. Standard definitions[14] of sensitivity, specificity, and predictive values of positive and negative tests were used to determine the ability of tumor-associated antigens to discriminate the presence or absence of metastatic disease.

Results

Pretherapy sera were obtained from 109 women with endometrial carcinoma before surgical staging or therapy. Ninety-seven patients (89%) had disease clinically localized to the uterus and cervix preoperatively, whereas 12 (11%) had advanced clinical stage disease (Table I). Elevations of CA 125 and CA 15-3 levels correlated with advanced clinical stage: 82% of patients with advanced disease had elevations of these tumor-associated antigen levels versus 11 and 14%, respectively, for patients with clinically localized disease ($p < 0.05$). Serum TAG 72 elevations were observed in 8% of patients with clinical stage I disease but only 36% of those with advanced disease (Table I). Elevations of CA 125 and CA 15-3 levels also were associated with advanced FIGO surgical stage ($p < 0.05$, Table I). However, elevated levels of these tumor-associated antigens were detected in 14% and 17% of 89 patients with disease histologically and cytologically confined to the uterus. Three of 33 patients with disease confined to the uterus who did not undergo lymph node sampling had elevated CA 125 levels; one had no residual

Table I. Serum tumor-associated antigen elevations versus clinical and surgical stage

	No.	CA 125* >35 U/ml (%)	TAG 72 >6 U/ml (%)	CA 15-3* >30 U/ml (%)
Clinical evaluation				
Confined to uterus	72	7 (10)	4 (6)	9 (13)
Cervical involvement	25	6 (24)	0	7 (28)
Advanced disease	12	10 (84)	5 (36)	10 (84)
FIGO surgical stage				
IA-IC	77	11 (14)	3 (4)	13 (17)
IIA-IIB	5	0	1 (20)	2 (40)
IIIA	8	0	0	0
IIIC	7	3 (43)	2 (29)	4 (57)
IVB	12	10 (83)	4 (33)	7 (58)

*$p < 0.05$ for clinical stage I plus II versus III-IV and surgical stage I plus II versus IIIA-IV.

Table II. Serum tumor-associated antigen elevations versus myometrial invasion and histologic grade: Disease confined to uterus and cervix

	No.*	CA 125 >35 U/ml (%)	TAG 72 >6 U/ml (%)	CA 15-3 >30 U/ml (%)
Myometrial invasion				
None	21	2 (10)	1 (5)	3 (14)
Inner third	40	5 (13)	2 (5)	7 (18)
Middle third	12	1 (9)	0	3 (27)
Outer third	16	3 (19)	1 (6)	2 (13)
Histologic grade				
1	35	3 (9)	2 (6)	4 (11)
2	38	5 (14)	1 (3)	9 (24)
3	16	3 (19)	1 (6)	2 (13)

*Includes seven patients with malignant peritoneal cytologic test results (stage IIIA).

invasive cancer in the hysterectomy specimen and the other two had inner myometrial invasion only.

The 89 patients with disease histologically confined to the uterus and cervix were further analyzed to determine whether extent of myometrial invasion or histologic differentiation correlated with elevation of the tumor-associated antigen levels (Table II). In general, there was not a significant relationship between elevated levels and either of these variables, although there was a trend toward elevated CA 125 levels among patients with deep myometrial invasion or histologic grade 3 lesions (Table II).

Comparison of the surgicopathologic extent of disease with preoperative serum tumor-associated antigen levels is displayed in Table III. Both CA 125 and CA 15-3 levels significantly correlated with surgicopathologic findings ($p < 0.05$, Table III). However, none of the seven patients with positive peritoneal cytologic findings and only one of three with histologically documented occult metastasis had preoperative elevations of any of the tumor-associated antigens (Table III).

The site and histologic grade of metastatic lesions appeared to be related to preoperative CA 125 elevations, although the differences were not statistically significant. Only three (38%) of eight patients with metastasis limited to the retroperitoneal nodes had elevated CA 125 levels versus 10 (91%) of 11 with carcinomatosis or extraabdominal metastasis. Similarly, CA 125 elevations occurred in three (38%) of eight women with grade 3 lesions compared with 10 (83%) of 12 patients with grade 1 or 2 lesions.

Serum TAG 72 and CA 15-3 levels were elevated in four patients with metastatic disease and normal CA 125 levels. However, these tumor-associated antigen levels were normal in nine and five patients, respectively, who had metastatic disease and elevated CA 125 levels. Either the TAG 72 or CA 15-3 levels were elevated in 11 (12%) of 89 with surgically localized disease, whereas four patients (4%) had coordinate elevations of CA 125 and one of the other tumor-associated antigen levels.

Eleven (12%) of 89 patients with disease histologically confined to the uterus and 13 (65%) of 20 with histologic evidence of extrauterine metastasis had elevated CA 125 levels (Table III). Therefore, elevations of serum CA 125 levels had a sensitivity of 65% and specificity of 88% with a positive predictive value of 54% and negative predictive value of 92% for predicting the presence or absence of extrauterine metastasis. The use

Table III. Serum tumor-associated antigen elevations versus extent of extrauterine metastasis

Extent of disease	*No.*	*CA 125* >35 U/ml (%)*	*TAG 72 >6 U/ml (%)*	*CA 15-3* >30 U/ml (%)*
Surgical stage I-II	82	11 (13)	4 (5)	15 (18)
Malignant peritoneal cytologic test results	7	0	0	0
Microscopic metastasis	3	0	1 (33)	0
Gross metastasis (>1 cm)	17	13 (76)	5 (29)	11 (65)

*$p < 0.05$, surgical stage I-II plus malignant cytologic test results versus microscopic plus gross metastasis.

of TAG 72 and CA 15-3 alone (Table III) or use of combinations of tumor-associated antigen levels did not significantly improve on the combined sensitivity and specificity of serum CA 125 alone. Elevations of one or more tumor-associated antigen levels had a sensitivity of 85% but specificity of only 71%, whereas coordinate elevations of CA 125 with either TAG 72 or CA 15-3 had a sensitivity of only 45% with specificity of 96%.

Preoperative clinical evaluation was compared with the surgicopathologic extent of disease and serum CA 125 elevations (Table IV). Although the CA 125 levels were elevated in only 11% of patients with clinically localized disease who were confirmed surgically to have localized disease and 90% of those with clinically advanced disease confirmed to have extrauterine metastasis, the preoperative CA 125 level did not distinguish between those patients with early clinical stage who had clinically occult extrauterine metastasis from those with benign adnexal masses who were found to have disease confined to the uterus on surgicopathologic evaluation (Table IV).

Table IV. Serum CA 125 elevations compared with clinical evaluation and surgical pathologic findings

	Surgical pathologic findings	
Clinical evaluation	*Confined to uterus and/or cervix**	*Extrauterine metastasis*
Localized to uterus and/or cervix	10/87 (11)†	4/10 (40)
Advanced disease	1/2 (50)	9/10 (90)

*Includes seven patients with malignant peritoneal cytologic results (FIGO stage IIIA).

†CA 125 elevations per total patients (%).

Comment

Recent studies by Duk et al.[4] and Patsner et al.[5] that reported elevation of circulating levels of CA 125 in women with endometrial adenocarcinomas prompted us to evaluate serum levels of tumor-associated antigens in our patient population. Duk et al.[5] found CA 125 elevations in 12 (67%) of 18 patients with surgically documented advanced disease. Patsner et al.[6] performed complete bilateral pelvic and paraaortic lymphadenectomy in all their patients with disease apparently confined to the uterus. They reported that elevation of CA 125 had a sensitivity of 90% and a specificity of 98% for discriminating surgically localized from surgically advanced disease.

Our findings do not support the routine use of preoperative serum CA 125 assays in women with endometrial carcinoma. Serum CA 125 elevations did correlate with clinical stage, surgical stage, and surgicopathologic extent of metastasis. However, we found an unacceptably high false-positive rate among patients with disease histologically confined to the uterus and cervix with a resultant positive predictive value of only 54% for elevations of CA 125 levels. Furthermore, none of those in the small subset of patients with occult extrauterine metastasis or malignant peritoneal cytology had elevations of CA 125. Finally, only 40% of patients with clinically occult but histologically confirmed metastasis had elevations of serum CA 125 levels before operation (Table IV); this is the group most likely to benefit from preoperative serum tumor-associated antigen level determinations.

We noted a false-positive rate of 12% for CA 125 elevations among women with endometrial carconima histologically confined to the uterus and cervix but were not able to demonstrate any difference by histologic grade or depth of myometrial invasion. It is unlikely that false-positive elevations resulted from omitting pelvic and paraaortic lymphadenectomy in 33 of our patients. The three "false-positive" results in this group occurred in patients at very low risk (less than 5%) for extrauterine metastasis.[2] Although the majority of cases of endometrial carcinoma express CA 125,[4,6] the mechanism for entry of the antigen into peripheral circu-

lation is unknown. It is assumed that CA 125 from endometrial lesions is shed into the endometrial cavity and the antigen gains access to the circulation through lymphatic drainage.[4] Elevations of serum CA 125 levels in women with endometrial carcinomas could be a result of both increased expression of CA 125 and breakdown of normal tissue barriers.

Duk et al.[4] suggested that elevated CA 125 levels in patients with early stage disease might identify those at risk for recurrence. This may reflect occult metastasis not detected by routine surgical staging techniques; however, all our study patients underwent upper abdominal exploration including peritoneal cytology and omental biopsy. Our follow-up data are not mature enough to confirm the prognostic importance of initial preoperative CA 125 elevations in patients with disease histologically confined to the uterus, cervix, or both areas.

The identification of elevated serum CA 125 levels in 65% of patients with histologic evidence of metastasis raises the possibility of monitoring CA 125 levels during therapy of those who have no measurable disease after surgical therapy. Duk et al.[4] reported a significant correlation between serial CA 125 levels and the clinical course of endometrial cancer in a small group of patients. Although many reports have confirmed the utility of serial CA 125 levels in monitoring of patients with ovarian carcinoma,[7, 15, 16] the role for monitoring serial CA 125 levels in women with endometrial carcinomas has not be established.

The two other tumor-associated antigen assays selected for this study, TAG 72 and CA 15-3, were chosen on the basis of several attributes. Both antigens are detected by monoclonal antibodies originally generated against membrane-enriched fractions of human breast carcinomas[9-13] but are also expressed by other types of benign and malignant human tissues. Tumor-associated glycoprotein defined by the monoclonal antibody B72.3 is expressed by the majority of carcinomas from a variety of primary sites, including endometrial adenocarcinomas.[10, 11, 17, 18] This antigen is also expressed by benign secretory endometrium.[17, 18] The shed antigen can be detected in the sera of women with malignant pelvic masses.[19] The DF-3 antigen (CA 15-3) is expressed by a variety of malignancies and levels are elevated in the sera of the majority of women with active breast[12] and ovarian carcinomas.[13, 19] Unlike TAG 72, DF-3 is expressed by benign endometrium in all phases of the menstrual cycle.[17] In a study of women with pelvic masses, we found that the use of these assays in conjunction with CA 125 assays improved the specificity for discriminating between benign and malignant masses compared with CA 125 alone.[18]

In this study CA 15-3 elevations correlated with clinical and surgicopathologic advanced endometrial carcinomas but had a higher false-positive rate than CA 125 levels. The TAG 72 assay had a much lower sensitivity for extrauterine disease than either CA 125 or CA 15-3. When these two tumor-associated antigen assays were used in conjunction with CA 125 levels, there was no significant improvement in the combined sensitivity or specificity for predicting the presence or absence of extrauterine metastasis versus CA 125 values alone.

Our data suggest that preoperative determination of serum tumor-associated antigen levels is not helpful in the routine evaluation of women with endometrial carcinoma. In our experience it appears that these tumor-associated antigen assays lack the sensitivity and specificity to reliably predict subclinical metastatic disease, although elevated levels frequently occur in patients with bulky metstasis.

REFERENCES

1. FIGO. FIGO—1988 revision. Gynecol Oncol 1989;35:125-6.
2. Creasman WT, Morrow CP, Bundy BN, Homesley HD, Graham JE, Heller PB. Surgical pathologic spread patterns of endometrial cancer. Cancer 1987;60:2035-41.
3. Niloff JM, Klug T, Schaetzl E, Zurawski R, Knapp RC, Bast RC. Elevation of serum CA 125 in carcinomas of fallopian tube, endometrium and endocervix. AM J OBSTET GYNECOL 1984;148:1057-8.
4. Duk JM, Aalders JG, Fleuren GJ, de Bruijn HWA. CA 125: a useful marker in endometrial carcinoma. AM J OBSTET GYNECOL 1986;155:1097-1102.
5. Patsner B, Mann WJ, Cohen H, Loesch M. Predictive value of preoperative serum CA 125 levels in clinically localized and advanced endometrial carcinoma. AM J OBSTET GYNECOL 1988;158:399-402.
6. Berchuck A, Soisson AP, Clarke-Pearson DL, et al. Cellular expression of CA 125 and metastatic potential of endometrial adenocarcinoma. Cancer Res 1989;49:2091-5.
7. Bast RC, Klug TL, St. John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian carcinoma. N Engl J Med 1983;309:883-7.
8. Niloff JM, Bast RC Jr, Schaetzel E, Reynolds C. CA 125 antigen levels in obstetrics and gynecologic patients. Obstet Gynecol 1984;64:703-7.
9. Nuti M, Teramoto YA, Mariani-Costanti R, Horan Hand P, Colcher D, Schlom J. A monoclonal antibody (B72.3) defines patterns of distribution of a novel tumor-associated antigen in human mammary cell populations. Int J Cancer 1982;29:539-45.
10. Thor A, Gorstein F, Ohuchi N, Szpak CA, Johnston WW, Schlom J. Tumor-associated glycoprotein (TAG-72) in ovarian carcinomas defined by monoclonal antibody B72.3. JNCI 1986;76:995-1006.
11. Colcher D, Horan Hand P, Nuti M, Schlom J. Differential binding to human mammary and non-mammary tumors of monoclonal antibodies reactive with carcinomembryonic antigen. Cancer Invest 1983;1:127-38.
12. Hayes DF, Sekine H, Ohno T, Keefe AR, Kufe DW. Detection of circulating plasma DF3 antigen levels in breast cancer patients. J Clin Invest 1985;75:1671-8.
13. Sekin H, Hayes DF, Ohno T, et al. Circulating DF3 and CA 125 antigen levels in serum from patients with epithelial ovarian carcinoma. J Clin Oncol 1985;3:1355-63.
14. Zar JH, ed. Biostatistical analysis. Englewood Cliffs, New Jersey: Prentice-Hall, 1984.

15. Kivinen S, Kuoppala T, Leppilampi M, Vuori J, Kauppila A. Tumor-associated antigen CA 125 before and during the treatment of ovarian carcinoma. Obstet Gynecol 1986; 67:468-72.
16. Lavin PT, Knapp RC, Malkasian G, Whitney CW, Berek JC, Bast RC, Jr. CA 125 for the monitoring of ovarian carcinoma during primary therapy. Obstet Gynecol 1987; 69:223-7.
17. Thor A, Viglione MJ, Muraro R, Ohuchi N, Schlom J, Gorstein F. Monoclonal antibody B72.3 reactivity with human endometrium: study of normal and malignant tissues. Int J Gynecol Pathol 1987;6:235-47.
18. Soisson AP, Berchuck A, Lessey BA, et al. Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels. Am J Obstet Gynecol 1989;161:1258-63.
19. Soper JT, Hunter VJ, Daly L, Tanner M, Creasman WT, Bast RC, Jr. Preoperative serum tumor-associated antigen levels in women with pelvic masses. Obstet Gynecol 1990; 75:249-54.

Maternal serum α-fetoprotein levels in pregnancies complicated by diabetes: Implications for screening programs

Alice O. Martin, PhD,[a] Lisa M. Dempsey, MS,[a] John Minogue, DMin,[a] Kiang Liu, PhD,[b] James Keller, MD,[a] Ralph Tamura, MD,[a] and Norbert Freinkel, MD[c, d, e]
Chicago, Illinois

Maternal serum α-fetoprotein may be reduced in diabetic pregnancies, but the association with elevated glycosylated hemoglobin has been controversial. We tested the hypothesis that reductions in maternal serum α-fetoprotein may reflect the same phenomena that can also impair normal rates of embryo growth in the presence of poorly compensated maternal diabetes. If so, associations would be expected among maternal serum α-fetoprotein, embryo rates of growth, and levels of glycosylated hemoglobin reflective of regulation of maternal diabetes during the period of organogenesis. We found maternal serum α-fetoprotein levels in 93 pregnant patients with diabetes to be negatively associated with the earliest (4 to 12 weeks) glycosylated hemoglobin determinations. At glycosylated hemoglobin values >9.6% (which approximates the upper quartile), all maternal serum α-fetoprotein values fell below the median for patients without diabetes (below 0.8 multiple of the median after weight adjustment). Moreover, there was a trend for pregnancies with lower maternal serum α-fetoprotein levels and higher glycosylated hemoglobin values to also demonstrate early fetal growth delay as measured by ultrasonography. (Am J Obstet Gynecol 1990;163:1209-16.)

Key words: Diabetes, maternal serum α-fetoprotein, glycosylated hemoglobin, fetal growth, neural tube defects

Ultrasonic monitoring from weeks 7 to 14 has indicated that delayed growth during early development may occur in some pregnancies complicated by diabetes.[1-3] The magnitude of the delay in growth has been correlated with the degree of antecedent and prevailing disturbance in maternal metabolism as gauged by elevations in glycosylated hemoglobin at that time.[4] The retarding effect of poorly regulated maternal diabetes on embryo growth has also been confirmed by direct analyses of conceptuses excised at the early postimplanation stage from rats rendered experimentally diabetic by the administration of streptozocin before conception.[5] These findings have suggested that aberrant fuel metabolism in the mother can compromise normal developmental timetables, and are consistent with the hypothesis of "fuel-mediated teratogenesis" proposed by Freinkel.[6, 7] The relationships may also be relevant to the recent reports of lower values for maternal serum α-fetoprotein (AFP) in diabetic pregnancies,[8, 9] and to

From the Departments of Obstetrics and Gynecology,[a] Community Health and Preventive Medicine,[b] Medicine,[c] and Molecular Biology[d] and the Center for Endocrinology, Metabolism and Nutrition,[e] Northwestern University Medical School.
Supported in part by the National Institutes of Health grants MRP-HD-11021, HD-19070, DK-10699, RR-48, and training grant DK-07169.
Received for publication August 29, 1989; revised May 7, 1990; accepted June 2, 1990.
Reprint requests: Alice O. Martin, PhD, Arnold, White and Durkee, 800 Quaker Tower, 321 N. Clark, Chicago, IL 60610.
6/1/22867

the conflicting literature concerning the magnitude and temporal aspects of the association between maternal serum AFP and maternal glycosylated hemoglobin.[10-12]

Correction factors linked to glycosylated hemoglobin have been proposed for the observed maternal serum AFP values in pregnancies complicated by diabetes on the assumption of some linear relationship.[11] It has also been suggested that glycosylated hemoglobin levels be checked in women with diabetes and that adjustments only be made on maternal serum AFP from women whose diabetes is not well controlled, rather than correcting maternal serum AFP values from all pregnant women with insulin-dependent diabetes with low maternal serum AFP values.[10] We believe that these corrections may belie the complexity of the true relationships between developmental events, glycosylated hemoglobin, and maternal serum AFP and note that application of such corrections has not been shown to increase sensitivity and specificity. If the sites for maternal serum AFP production are regulated by the same fuel-related phenomena that determines retardation of embryo growth, an attenuation of maternal serum AFP levels might be anticipated.[10] Moreover, the degree of attenuation should bear some relationship to the disturbance in maternal metabolism during embryogenesis (as judged by glycosylated hemoglobin in early pregnancy) and by the impact of the faulty metabolism on the manifest growth of the conceptus shortly thereafter (as judged by early ultrasonography). Because glycosylated hemoglobin reflects blood glucose levels 4 to 8 weeks before sampling, values taken after 12 weeks of pregnancy may not reveal these associations.

To test the above hypotheses, we determined whether (1) maternal serum AFP values are associated with the degree of diabetic control during embryogenesis, as measured by glycosylated hemoglobin in early in pregnancy (4 to 12 weeks) rather than closer to the time of the sampling for the estimation of maternal serum AFP (14 to 24 weeks) and (2) whether delayed fetal growth is correlated with maternal serum AFP and the early glycosylated hemoglobin values.

Material and methods

Sample. Data were collected for patients seen at Prentice Women's Hospital and Maternity Center of Northwestern Memorial Hospital. (The project had the approval of the Human Subjects Committee of Northwestern University.) Maternal serum AFP screening has been routinely offered since 1984.[13] We examined records from 1985 to 1988 of our patients with diabetes and selected all women with diabetes who also had maternal serum AFP screening. Only the latest pregnancy of each woman was included in the sample so that each could be considered independent for purposes of statistical analysis. Multiple pregnancies were excluded. We selected two of the glycosylated hemoglobin determinations available for each woman: (1) the earliest value obtained in the most recent pregnancy (67% between 4 and 12 weeks) as a measure of diabetes control before entering our program; and (2) that value obtained closest in time to when the maternal serum AFP sample was taken (96% between 14 and 24 weeks). For nine women, only one glycosylated hemoglobin value was available; this value was used as both an "early" and "closest" one. Each patient's clinical class was noted. The outcome of all pregnancies was determined. The 93 patients with diabetes (White classes B to R) ascertained in this manner were compared with 4674 nondiabetic controls screened for maternal serum AFP during the same period. Information on maternal weight at the time that maternal serum AFP was measured was available for 51 diabetic and 1129 nondiabetic pregnant women.

Estimation of gestational age for maternal serum AFP standardization and glycosylated hemoglobin selection. Information on gestational age obtained from patient records included last menstrual period and all dates determined by ultrasonography. Gestational age on the basis of consideration of all available data was calculated according to the specifications of Depp[14] and used for standardization of maternal serum AFP and for selecting the two glycosylated hemoglobin values used in our study.

Maternal serum AFP values. The laboratory values of maternal serum AFP were expressed as nanograms per milliliter for each week of gestation using the Abbott polyclonal enzyme immunoassay (Abbott Labs., North Chicago) for 71 patients. An additional 22 patients had maternal serum AFP determinations by the Kallestad AFP/OB radioimmunoassay kit (Kallestad Laboratories Inc., Austin, Tex.) during 1985.[13] Multiples of the median were computed from internal standards based on maternal serum AFP distributions computed for each gestational week for each assay. The distributions were also examined after application of weight corrections using the formula for expected multiples of the median $= 10^{[0.2658 - 0.00188\,(\mathrm{lbs})]}$.[14]

Glycosylated hemoglobin levels. Glycosylated hemoglobin determinations in the hospital laboratory are made at regular intervals during pregnancies of diabetic women at our center with gel electrophoresis (Corning, Medfield, Mass.). Mean values for nongravid, nondiabetic individuals have averaged 6.2% with a standard deviation of 0.85% (range, 5.3% to 7.2%).

Fetal growth delay. For determination of fetal growth delay, gestational age based on last menstrual period was compared with that based on ultrasonography. A Kallestad diagnostic wheel was used to convert last menstrual period to gestational days at the time of ultrasound dating. The ultrasonographic estimate

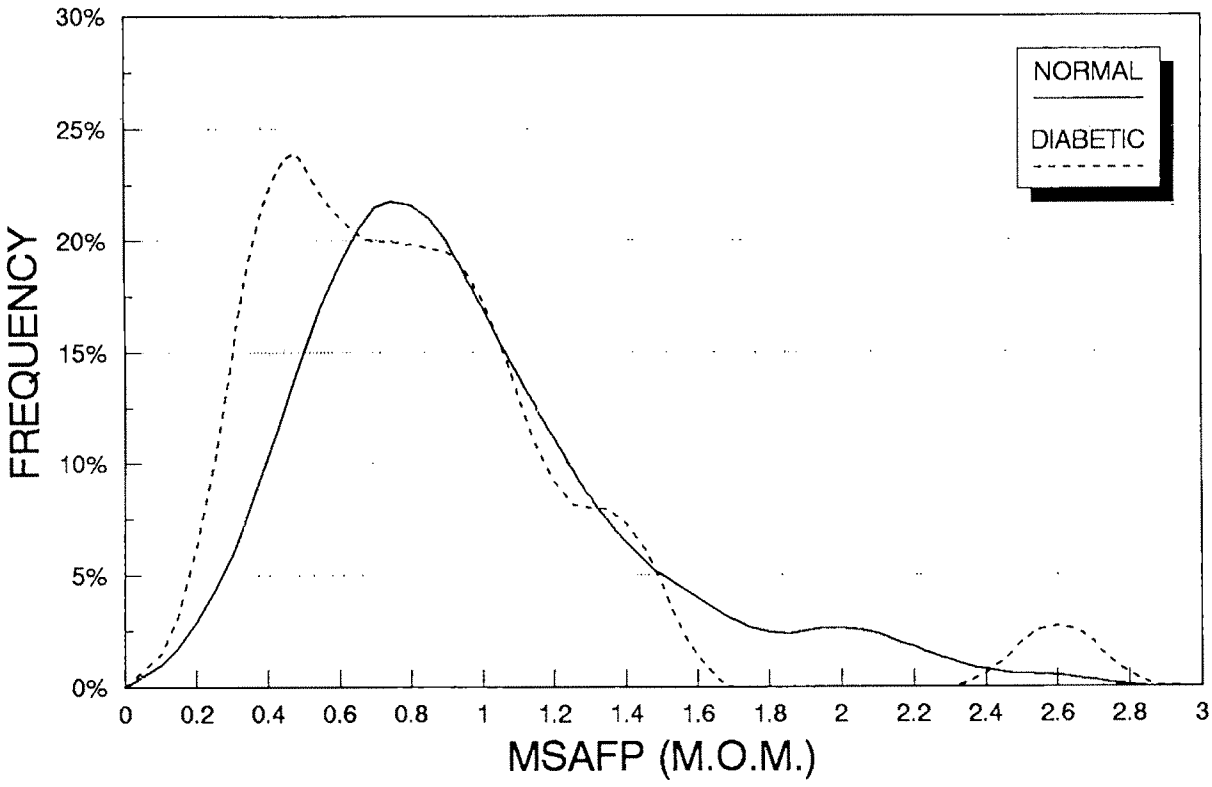


Fig. 1A. Distribution of maternal serum AFP values in diabetic women (n = 93) compared with nondiabetic women (n = 4674). Units are multiples of median.

of gestational age was obtained by the crown-rump length[15] closest to 14 weeks (range, 5 to 13 weeks). This value was the average of three measurements made with dynamic-image ultrasonography by described methods.[16] Forty-one of the 93 patients with diabetes had both last menstrual period and crown-rump length information available. These two estimates in days were then compared.

Fetal growth delay in these 41 patients was defined by two separate criteria based on whether ultrasonography provided lower estimates than last menstrual period (1) by ≥6 days, the criteria used by Pedersen et al.[2,4] for early fetal growth delay; and (2) by ≥4 days, criteria accepted by ultrasonologists in our institution as evidence of delay. Means for glycosylated hemoglobin and maternal serum AFP (multiples of the median) were compared separately in those two groups versus all others. In addition, correlation coefficients were determined between (glycosylated hemoglobin and maternal serum AFP) and all the differences in gestational age estimates, regardless of direction or magnitude.

Statistical analysis. The distribution of maternal serum AFP in women with diabetes was compared with that of women without diabetes by χ^2 analysis. A comparison was also made after adjusting maternal serum AFP for weight.

Product-moment correlation coefficients were computed between glycosylated hemoglobin and maternal serum AFP for all diabetics with either the earliest glycosylated hemoglobin assay, or the value of glycosylated hemoglobin obtained closest to when the maternal serum AFP sample was obtained. Correlations were also computed between glycosylated hemoglobin or maternal serum AFP and all differences in gestational age estimates regardless of direction or magnitude. Mean glycosylated hemoglobin (early) and maternal serum AFP levels for fetuses with growth delay were compared with all others by t tests. These comparisons were made separately using criteria for delay of ≥6 days and ≥4 days. These two comparisons are not independent.

Maternal serum AFP distribution in women with diabetes. There is a significant shift in the distribution of maternal serum AFP in diabetic women toward lower values ($\chi^2_5 = 11.07$, $p < 0.05$) (Fig. 1A). Weight adjustment improved the fit of diabetic to nondiabetic distributions ($\chi^2_5 = 7.24$, $p > 0.05$) (Fig. 1B).

Association between maternal serum AFP and glycosylated hemoglobin. By inspection, no linear association was readily apparent between maternal serum AFP and either glycosylated hemoglobin value, although the correlation coefficient is significant ($p <$ 0.05) for the earliest glycosylated hemoglobin (r =

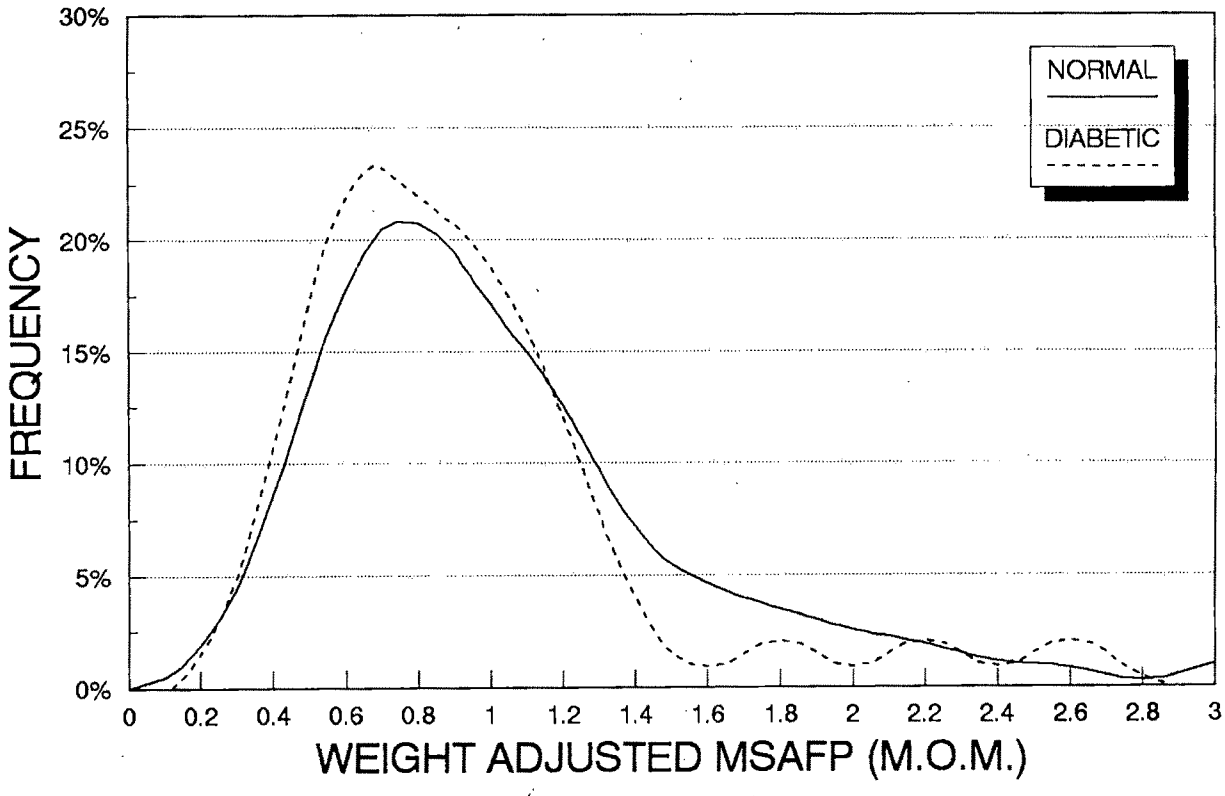


Fig. 1B. Distribution of maternal serum AFP values after adjustment of weight (diabetic, $n = 51$; nondiabetic, $n = 1120$).

-0.20, $r = -0.25$ after weight adjustment). The nature of the association becomes clearer when the highest values (upper quartile) of glycosylated hemoglobin are compared with the other three quartiles. Values for maternal serum AFP are scattered about the median for nondiabetic subjects up to a value for the *early* glycosylated hemoglobin of approximately 9.6% (Fig. 2A). (This would be approximately 4 SDs above the mean for normal, nonpregnant persons, according to glycosylated hemoglobin determinations as performed in our hospital laboratory.) Above this level, all maternal serum AFP values fell below the median for nondiabetic patients. This accounts for approximately 25% of the diabetic population with a mean maternal serum AFP in that quartile of 0.68 in contrast to mean values of 0.84, 0.82, and 0.86 in the other quartiles. This association was noted with either assays of maternal serum AFP (Kallestad or Abbott) and persisted after adjusting maternal serum AFP for weight (Fig. 2B). This relationship was not evident for glycosylated hemoglobin values obtained closer to the time of maternal serum AFP sampling (i.e., later glycosylated hemoglobin).

Fetal growth delay, maternal serum AFP. Our subset of 41 patients for whom we had also obtained early estimates of crown-rump length by ultrasonography was representative of our total patient population of 93 in exhibiting a similar association between glycosylated hemoglobin and maternal serum AFP ($r = -0.31$). Estimates of embryo size by early ultrasonography compared with gestational age that is based on last menstrual period show that last menstrual period estimated dates are generally greater than those based on ultrasonography (29/41). Nonetheless, when we used "at least 6 days" (32% of our sample)[2,4] or "at least 4 days" (i.e., 43% of our sample) as the criteria for "delayed growth," we found that this population displayed a general shift toward lower values for maternal serum AFP and higher values for glycosylated hemoglobin (Table I). Although these results are in the direction predicted by our hypothesis, the differences between mean maternal serum AFP and glycosylated hemoglobin of those with "delayed growth" versus those with "normal growth" were not statistically significant by t tests. This is probably a result of small sample sizes, and a larger series may be necessary for definitive configuration.

Although anomalies were recorded in nine of the 91 live-born infants, no neural tube defects were found. The only major malformations occurred in two infants, one with a congenital heart defect (univentricular heart with tricuspid artesia; this infant also had a hemivertebra and scoliosis, maternal serum AFP = 0.76, and

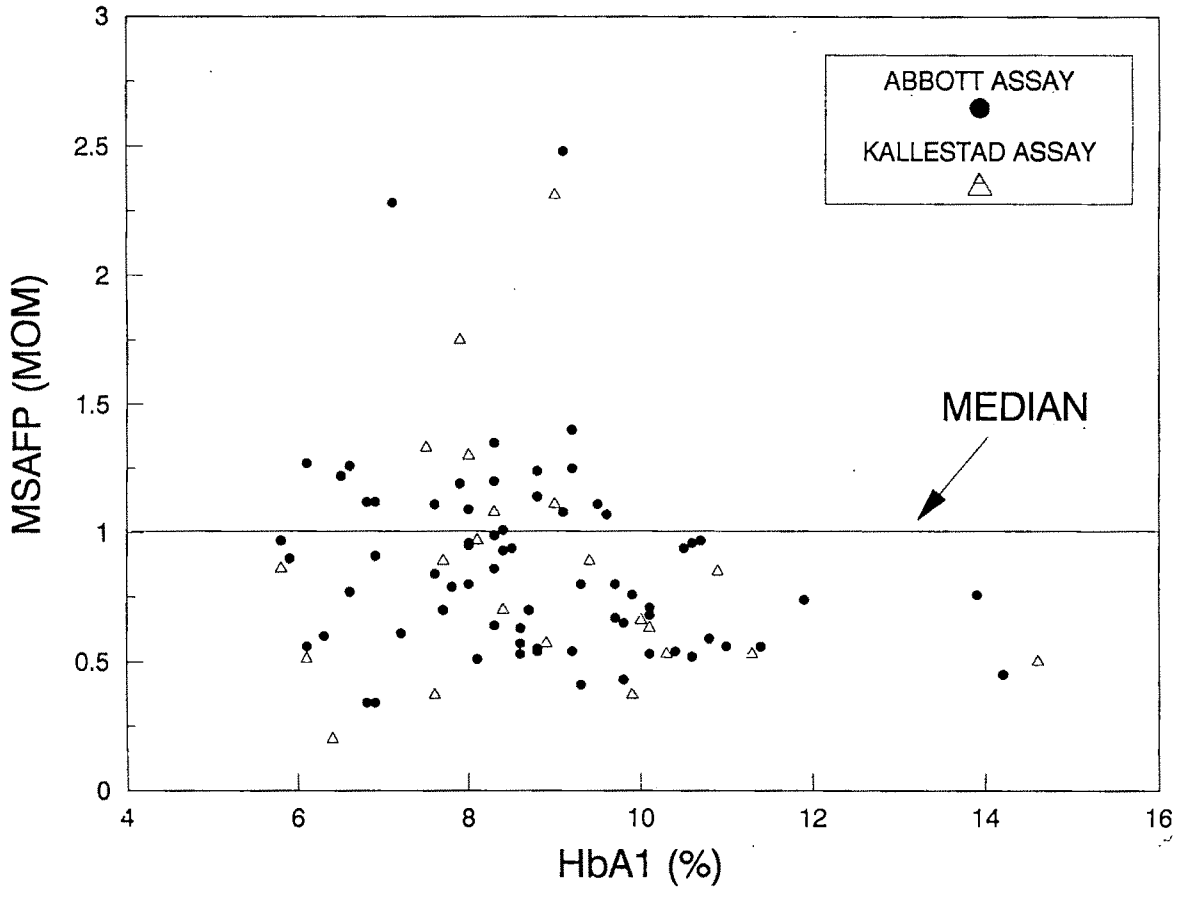


Fig. 2A. Bivariate distribution of maternal serum AFP (multiples of median) and earliest glycosylated hemoglobin levels in 93 diabetic women (three are overlapping values). Values obtained with Abbott maternal serum AFP assay are distinguished from those with Kallestad assay.

early glycosylated hemoglobin = 13.9%); and one had a cystic adenomatoid malformation of the lung (maternal serum AFP = 0.43, and early glycosylated hemoglobin = 9.8%). One infant of a 44-year-old mother had Down syndrome (maternal serum AFP = 0.53, glycosylated hemoglobin = 10.1%). Too few anomalies were found in the subset with data available on early ultrasonography glycosylated hemoglobin, and maternal serum AFP to perform formal analysis of the predictive value of these parameters, although both infants with major birth defects were associated with glycosylated hemoglobin values >4 SDs from the mean of normal values, and with maternal serum AFP below the median.

Comment

We examined the relationship between maternal serum AFP and glycosylated hemoglobin levels in 93 pregnancies complicated by diabetes and the associated ultrasonographic patterns of early fetal growth in 41 of these cases. We found that reduction in maternal serum AFP values occurs when the metabolic disturbances are most pronounced during early pregnancy, as judged by glycosylated hemoglobin values obtained when the patient first entered our program (67% during weeks 4 through 12). These values reflect the regulation of maternal diabetes during the periconceptual interval when embryogenesis takes place. At glycosylated hemoglobin values of approximately 9.6% (that is, >4 SDs over the normal mean values in our hospital laboratory) and within the upper quartile for the diabetic gravid patient in this series, all maternal serum AFP values were below the median (below 0.8 multiples of the median after adjustment for weight). Later values for glycosylated hemoglobin values obtained near the time of maternal serum AFP sampling were not as informative. The latter are less likely to reflect the regulation of maternal metabolism that prevailed during embryogenesis. At present we cannot explain the temporal discrepancies in our study and the findings of Reece et al.[10] of "inverse correlation between maternal serum AFP and the concentration of glycosylated hemoglobin when both assays were done within 6 weeks of each other (n = 47) . . . but not when glycosylated

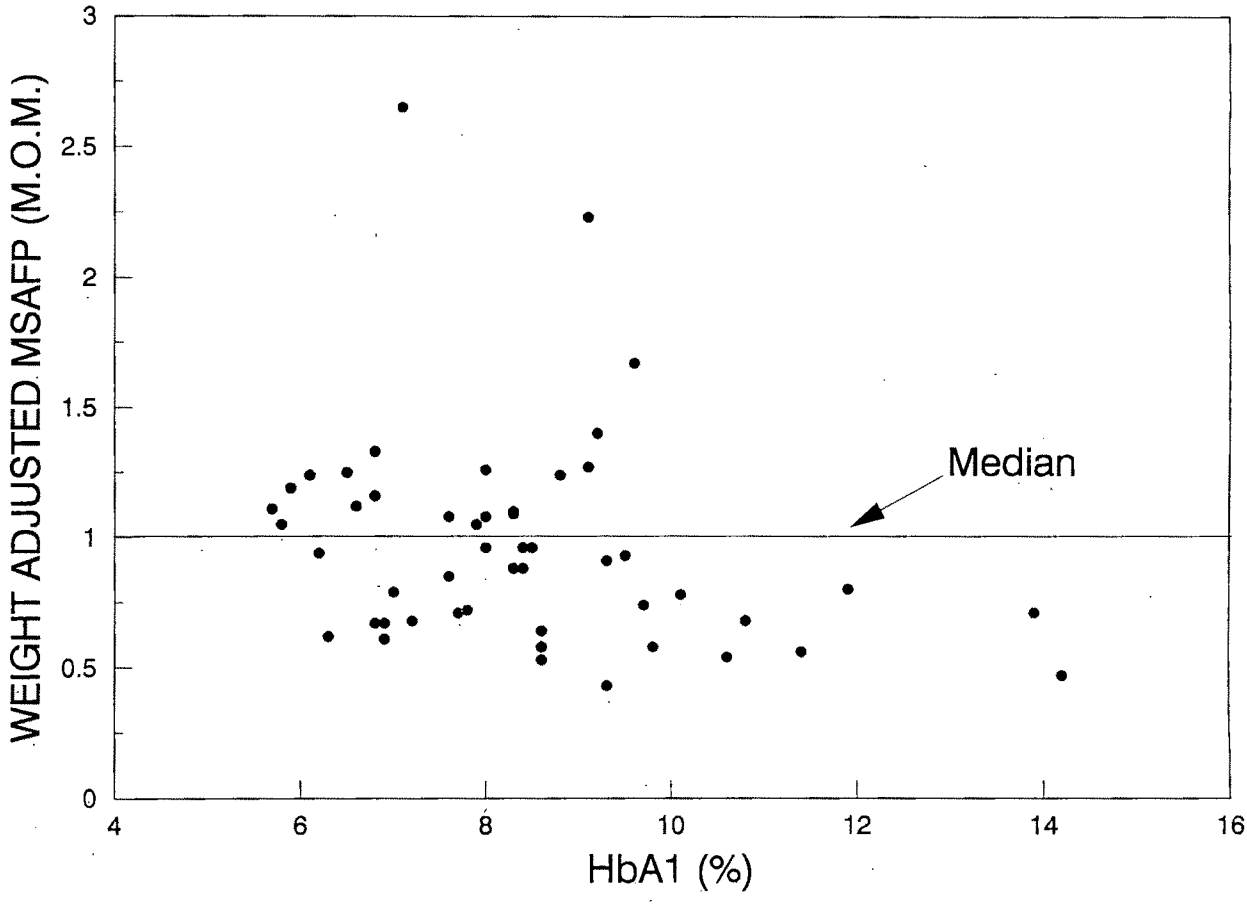


Fig. 2B. Distribution of glycosylated hemoglobin and maternal serum AFP after adjusting the latter for maternal weight. All of these assays were performed with the Abbott test kit (n = 51).

Table I. Glycosylated hemoglobin and maternal serum AFP in fetuses exhibiting delayed fetal growth

			Glycosylated hemoglobin (%)		*Maternal serum AFP*	
	n	*%*	*Mean ± SE*	*Range*	*Mean ± SE*	*Range*
Gestational age (LMP − US) shows delay of ≥6 days*						
6 days	13	32	9.16 ± 0.59	6.6-14.2	0.72 ± 0.08	0.34-1.21
Other	28	68	8.89 ± 0.25	6.1-11.9	0.82 ± 0.05	0.41-1.42
TOTAL	41					
Gestational age (LMP − US) shows delay of ≥4 days†						
4 days	18	43	9.26 ± 0.43	6.6-14.2	0.71 ± 0.06	0.34-1.21
Other	23	57	8.75 ± 0.28	6.1-11.9	0.86 ± 0.06	0.41-1.42
TOTAL	41					

LMP, Last menstrual period; *US,* ultrasonography.
*Criteria of Pedersen et al.[2, 4]
†Northwestern criteria.

hemoglobin was measured in the first 2 months of gestation (n = 26)."

In view of these findings, discrepancies in glycosylated hemoglobin (or hemoglobin A_{1c}) and maternal serum AFP associations among several of the studies may be explained by the proportion of women with high glycosylated hemoglobin (or hemoglobin A_{1c}) in these reports, the time in gestation at which the specimens for glycosylated hemoglobin were obtained, and the laboratory values defining the upper quartile. Negative correlations are unlikely to appear if there are no increased levels of glycosylated hemoglobin, as is to have been the case likely in the nondiabetic pregnancies in which Baumgarten and Robinson[11] found no asso-

ciations. Because the association in our study does not appear to be linear, statistical tests that assume linearity are questionable, although the correlation coefficient was significant in both our series and the studies by Baumgarten and Robinson.[11] Indeed, the apparent discrepancies between the finding by Greene et al.[12] of no association and the negative associations that we and others have observed, may be reconciled by noting that the data of Greene et al. also show a decrease in maternal serum AFP in the upper quartile of glycosylated hemoglobin, >9.4% as evident in our data (means = 0.84, 0.82, 0.86, 0.69). Moreover, in the scattergram presented by Baumgarten and Robinson,[11] maternal serum AFP values are reduced in the two patients in their series whose values for glycosylated hemoglobin were >4 SDs above the mean value for their normal pregnant women.

The postulated interrelationship of maternal serum AFP and glycosylated hemoglobin has particular significance with regard to the use of maternal serum AFP as a predictive index of birth defects, fetal distress, and chromosomal aberrations. It has been shown that the incidence of birth defects is increased in fetuses with early growth delay,[2, 3] presumably when metabolic abnormalities are at their highest level.[4] If so, a biphasic consequence might result. The delayed growth would *reduce* maternal serum AFP levels, in some way linked to the factors responsible for growth retardation, and the attendant birth defects or fetal distress would *elevate* maternal serum AFP values as a consequence of lesions at the site of the birth defect. Thus a normalization of maternal serum AFP might result as a consequence of these two opposing vectors. In view of these diverging possibilities, some estimate of embryo size as judged by early ultrasonography may be useful as an additional parameter for interpreting maternal serum AFP values, especially when the likelihood of birth defects in early diabetic pregnancy is greatest.

Justification for adjustment of maternal serum AFP levels for weight, race, diabetic status, or glycosylated hemoglobin level has been based on observations that maternal serum AFP distributions vary according to those factors. However, improved sensitivity and specificity must be established as resulting from application of these proposed corrections before they are applied for clinical screening. Indeed, because the prevalence of neural tube defects varies as a function of some of these variables, (e.g., maternal diabetes[8, 9]), and because the distribution of maternal serum AFP in affected fetuses when their mothers are classified by diabetic status is unknown, applying corrections could reduce sensitivity. This untoward outcome has been suggested for nondiabetic pregnancies using weight adjustments.[17]

The only major malformations noted in our series were a congenital heart defect and a hemivertebra and scoliosis in one infant, and a tumor of the lung in another. No neural tube defects were noted. Although maternal serum AFP values were low in both those infants and glycosylated hemoglobin levels were elevated well in excess of 4 SDs from the normal mean in our hospital laboratory, the numbers were too small for formal analysis of the interrelationship.

We observed a trend of both low maternal serum AFP and high glycosylated hemoglobin values in pregnancies that exhibited fetal growth delay, supporting our hypothesis that altered maternal metabolism effects on fetal growth are reflected in lowered maternal serum AFP. Larger sample sizes including more fetuses with anomalies and delayed growth will be needed to elucidate the overall associations. In the interim we suggest rather than applying corrections to maternal serum AFP, other than perhaps for weight, that special attention be paid to pregnancies with low unadjusted maternal serum AFP, or with elevated glycosylated hemoglobin values, or both, in the first trimester. If the glycosylated hemoglobin data are unavailable for that period, the maternal serum AFP level after 15 weeks may be a valuable indicator of earlier lack of control during embryogenesis and organogenesis, based on the negative association we report. Determination of glycosylated hemoglobin in the second trimester may not be a useful adjuvant because glycosylated hemoglobin reflects control 4 to 8 weeks earlier, which in this case may not be indicative of developmental problems that would have occurred earlier. Early determination of crown-rump length by ultrasonography may be recommended as an additional parameter for interpretation of maternal serum AFP. If further experience supports our preliminary findings, the triad of maternal serum AFP, glycosylated hemoglobin, and early ultrasound, considered jointly, may well provide the most accurate determination of teratogenic risk in pregnancies of diabetic women complicated by diabetes mellitus.

We thank Robert Mardur, MD, Jayme Borensztajn, MD, and the Department of Pathology for performing the laboratory assays, and Patricia Lathion, who assisted in the preparation of this article.

REFERENCES

1. Pedersen JF, Molsted-Pedersen L. Early growth retardation in diabetic pregnancy. Br Med J 1979;1:18-9.
2. Pedersen JF, Molsted-Pedersen L. Early fetal growth delay detected by ultrasound marks increased risk of congenital malformation in diabetic pregnancy. Br Med J 1981; 283:269-71.
3. Tchobroutsky C, Breart GL, Rambaud DC, Henrion R. Correlation between fetal defects and early growth delay observed by ultrasound. Lancet 1985;1:706-7.
4. Pedersen JF, Molsted-Pedersen L, Mortensen HB. Fetal growth delay and maternal hemoglobin A_{1c} in early diabetic pregnancy. Obstet Gynecol 1984;64:351-2.
5. Eriksson UJ, Lewis NJ, Freinkel N. Growth retardation

during early organogenesis in embryos of experimentally diabetic rats. Diabetes 1984;33:281-4.
6. Freinkel N, Banting Lecture 1980. Of pregnancy and progeny. Diabetes 1980;29:1023-35.
7. Freinkel N. Diabetic embryopathy and fuel-mediated organ teratogenesis: lessons from animal models. Horm Metab Res 1988;20:463-75.
8. Wald NJ, Cuckle H, Borcham J, Stirrat GM, Turnbull A. Maternal serum alpha-fetoprotein and diabetes mellitus. Br J Obstet Gynaecol 1979;86:101-5.
9. Milunsky A, Alpert E, Kitzmiller JL, Younger MD, Neff RK. Prenatal diagnosis of neural tube defects. VIII. The importance of serum alpha-fetoprotein screening in diabetic pregnant women. AM J OBSTET GYNECOL 1982; 142:1030-2.
10. Reece EA, Davis N, Mahoney MJ, Baumgarten A. Maternal serum alpha-fetoprotein in diabetic pregnancy: correlation with blood glucose control. Lancet 1987; 2:275.
11. Baumgarten A, Robinson J. Prospective study of an inverse relationship between maternal glycosylated hemoglobin and serum alpha-fetoprotein concentrations in pregnant women with diabetes. AM J OBSTET GYNECOL 1988;159:77-81.
12. Greene MF, Haddow JE, Palomaki GE, Knight GJ. Maternal serum alpha-fetoprotein levels in diabetic pregnancies. Lancet 1988;2:345-6.
13. Simpson JL, Baum LD, Marder R, Elias S, Ober C, Martin AO. Maternal serum α-fetoprotein screening: low and high values for detection of genetic abnormalities. AM J OBSTET GYNECOL 1986;155:593-7.
14. Depp R. Dynamics of fetal growth. In: Sabbagha R, ed. Diagnostic ultrasound applied to obstetrics and gynecology. 1st ed. Hagerstown, Maryland: Harper & Row, 1980:111-25.
15. MacGregor SN, Tamura RK, Sabbagha RE, Minogue JP, Gibson ME, Hoffman DI. Underestimation of gestational age by conventional crown-rump length dating curves. Obstet Gynecol 1987;70:344-8.
16. Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw-Hill, 1984:52-4.
17. Macri JN, Kasturi RV, Krantz DA, Koch KE. Maternal serum α-fetoprotein screening, maternal weight, and detection efficiency. AM J OBSTET GYNECOL 1986;155:758-60.

High infectious morbidity in pregnant women with insulin-dependent diabetes: An understated complication

Eric F. Stamler, MD,[a] Maria L. Cruz, MD,[b] Francis Mimouni, MD,[b] Barak Rosenn, MD,[a] Tariq Siddiqi, MD,[a] Jane Khoury, MS,[c] and Menachem Miodovnik, MD[a]

Cincinnati, Ohio

Patients with insulin-dependent diabetes are prone to infection, possibly related to poor metabolic control. Relative immune deficiency exists in pregnancy. We hypothesized that pregnant patients with insulin-dependent diabetes are at an increased risk for infection and that infection is related to poor glycemic control. We matched 65 pregnant women with insulin-dependent diabetes to 65 nondiabetic pregnant controls. At least one episode of infection before delivery occurred in 83% of the women with insulin-dependent diabetes (26% in control group). The rate of postpartum infection was five times higher in the group with insulin-dependent diabetes and they were susceptible to more kinds of infections. Although there was no overall difference among the indices of glycemic control, hemoglobin A_1 obtained before the infection was higher than during infection. We conclude that a high rate of infection exists in pregnant women with diabetes; infection and poor glycemic control may be associated, but it is unclear whether improvement in metabolic control will reduce this high infection rate. (Am J Obstet Gynecol 1990;163:1217-21.)

Key words: Diabetes, pregnancy, infection, glycohemoglobin

Patients with insulin-dependent diabetes are at high risk for infection.[1] Several deficiencies in the immune mechanism involving defective leukocyte and lymphocyte activity[2-6] may explain their propensity to infection. These deficiencies appear to be linked to poor glycemic control.[4-6]

Pregnancy is also generally thought to constitute a state of relative immune deficiency, specifically, impaired cell-mediated immunity.[7-10] Thus pregnancy in the patient with insulin-dependent diabetes is likely to represent an additional risk factor for infection.

This study was designed to test the hypotheses that (1) pregnant patients with insulin-dependent diabetes have a higher rate of antepartum and postpartum infections when compared with a pair-matched control population, and (2) high infection rates in pregnancy patients with insulin-dependent diabetes correlate with poor glycemic control. Specifically, glycemic control during pregnancy is related to infection in pregnancy and glycemic control in the third trimester is related to postpartum infection.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[a] the Division of Neonatology, Department of Pediatrics,[b] and the Division of Biostatistics, Department of Environmental Health,[c] Perinatal Research Institute, University of Cincinnati College of Medicine.

Supported in part by National Institutes of Health grants HD-11725, RR-00068, and HD-20748, the Children's Hospital Research Foundation, and the Gerber Product Company.

Received for publication February 13, 1990; revised May 14, 1990; accepted June 15, 1990.

Reprint requests. Menachem Miodovnik, MD, University of Cincinnati College of Medicine, 231 Bethesda Ave., Cincinnati, OH 45267-0526.

6/1/23237

Material and methods

In an interdisciplinary program at the University of Cincinnati designed to study the effects of glycemic control on the course and outcome of pregnancy in patients with insulin-dependent diabetes, 307 pregnancies in diabetic patients were prospectively followed from 1978 to 1988. To avoid possible biases and the confounding effect of preterm or prolonged rupture of membranes, labor, or vaginal deliveries on the incidence of infection, only 65 patients with insulin-dependent diabetes delivered by elective cesarean section for previous cesarean delivery were included in this study. According to White's classification, 16% of these patients with insulin-dependent diabetes were in class B, 33% were in class C, and 51% in class D to RT. The study group was retrospectively compared with a control group of 65 nondiabetic controls (normal glucose challenge at 28 weeks' gestation[11]) who were also delivered by repeat elective cesarean section. Control patients were randomly selected by computer after careful matching by gestation (± 2 weeks), parity, age (± 3 years), year of delivery, presence or absence of labor, and care by the same obstetrician (Table I). Complete medical and obstetric histories were recorded for each patient. Control patients were seen monthly until the thirty-sixth week of gestation, then weekly there-

Table I. Demographic data in diabetic and control populations†

	Diabetic (n = 65)	*Control (n = 65)*
Age (yr)	27.7 ± 0.57	28.4 ± 0.52
Gestation (wk)	37.0 ± 0.20	37.8 ± 0.26
Parity	2.2 ± 0.11	2.2 ± 0.09
White	59 (91%)	59 (91%)
Presence of labor at delivery	16 (25%)	16 (25%)

*Data expressed as mean ± SEM or number and percent.
†There were no significant differences between groups.

Table II. Infection rates in diabetic and control populations*

	Diabetic	*Control*	*Significance*
Antepartum			
At least one episode	54 (83%)	17 (26%)	$p < 0.01$
No. of episodes per woman	1.58 ± 0.14	0.34 ± 0.08	$p < 0.01$
Postpartum			
At least one infection	16 (24%)	3 (5%)	$p < 0.01$
No. of infections per woman	0.29 ± 0.07	0.05 ± 0.03	$p < 0.01$

*Data expressed as mean ± SEM or number and percent.

after. Because the nature of their disease, patients with insulin-dependent diabetes were seen every 1 to 2 weeks.

In patients with insulin-dependent diabetes, glycemic control was obtained with split-dosage regimens of insulin using both short- and intermediate-acting preparations in combination with dietary regulation. The goals of glycemic control were a fasting blood glucose concentation of <100 mg/dl and a 90-minute postprandial glucose concentration of <140 mg/dl. Patients were hospitalized when necessary to maintain these goals of glycemic control. Maternal blood hemoglobin A_1 concentrations were measured at entry and every 4 weeks using the Isolab column chromatography, with an interassay coefficient of variation of 7.2% and a normal nonpregnant range of 5.5% to 8.5%.[12] Preprandial or postprandial capillary glucose concentration were alternately measured at each weekly visit with a reflectance meter (Dextrometer, Ames Division, Miles Laboratories, Elkhard, Ind.). Results for fasting glucose (or postprandial glucose) were averaged for each trimester and expressed as mean fasting glucose (or postprandial glucose) per trimester.

Patients were evaluated for infection only if they were symptomatic. Infections were classified according to time of onset during pregnancy (antepartum vs postpartum), by system involved (urinary, genital, gastrointestinal, respiratory, specific viral syndromes, wound infection, and endometritis), and by specific infective agent (bacterial, protozoal, viral, fungal). The following criteria were required for the diagnosis of an acute infection: (1) culture of a specific organism from a relevant specimen or positive wet smear, potassium hydroxide preparation or Gram stain from a relevant specimen for the diagnosis of vaginitis, urinary tract infection or cystitis, or wound infection; (2) physical symptoms of either fever, rhinorrhea, cough, myalgia, or headache for the diagnosis of respiratory tract infections or presumed viral syndrome; (3) physical symptoms of diarrhea, vomiting, or both for the diagnosis of gastroenteritis; (4) presence of uterine tenderness and fever for the diagnosis of endometritis.

Data were analyzed with the SAS program (SAS Institute, Cary, N.C.). Continuous data were tested for normality and were analyzed, after appropriate transformation when necessary, by means of analysis of variance techniques and Student's t test. When transformation was not possible, nonparametric techniques were used; χ^2 or Fisher's exact test were used as tests for categoric variables. Logistic regression analysis was used to determine the combined effects of factors found to be significant in univariate analysis and to test their possible interaction. Data are expressed as mean ± SEM. A p value <0.05 was considered significant.

Results

Infection rates are presented in Table II. There were significantly more women in the group with insulin-dependent diabetes who had at least one episode of infection during pregnancy than women in the control group (83% vs 26%, $p < 0.01$). Postpartum infection rates were also significantly higher in the diabetic group (24% vs 5%, $p < 0.01$). The mean number of infectious episodes per women was also higher in the group with insulin-dependent diabetes in both the antepartum (1.58 ± 0.14 vs 0.34 ± 0.08), and postpartum (0.29 ± 0.07 vs 0.05 ± 0.03, $p < 0.01$ using Wilcoxon rank-sum).

The types of infection are presented in Tables III and IV. Women in the group with insulin-dependent diabetes demonstrated significantly higher rates of urogenital, respiratory tract, wound, and endometrial infections compared with the control group. Of the various infective agents tested, only Candida infection was found more frequently in the group with insulin-dependent diabetes ($p < 0.01$).

The relationship between infection in women with insulin-dependent diabetes and glycemic control is presented in Tables V and VI. There was no significant difference in the mean hemoglobin A_1, fasting glucose, and postprandial glucose measurements in each period between the group with infection in that trimester and

the group without infection in the same trimester in both the antepartum and postpartum periods.

To further define the temporal relationship between glycemic control and infection and to remove the possible confounding effect of repeated infection on hemoglobin A_1 concentrations, we analyzed hemoglobin A_1 concentrations in two ways. (1) The mean hemoglobin A_1 obtained before the first infectious episode in women with insulin-dependent diabetes in whom infection developed was compared with the mean hemoglobin A_1 of the same women obtained around the time of the infection (within 2 weeks before to 6 weeks after the infection), and was found to be significantly higher (9.5 ± 0.31 vs 8.5 ± 0.32, $n = 34$, $p < 0.01$). (2) The mean hemoglobin A_1 at 9 weeks was compared among three groups of women: (a) those who had no infection at 9 weeks but in whom infection subsequently developed between 10 to 20 weeks ($n = 18$); (b) those who had their first episode of infection later than 20 weeks' gestation ($n = 12$), and (c) those in whom antepartum infection never developed ($n = 9$). The mean ± SEM values were 9.72 ± 0.48, 9.42 ± 0.56, and 9.06 ± 0.50, respectively (no significant differences).

Comment

Our data show that patients with insulin-dependent diabetes are at high risk for infectious complications both during pregnancy and in the postpartum period. We cannot exclude the possibility that there could have been underreporting of infections in the control group, since women in this group were not seen at follow-up as frequently as women with insulin-dependent diabetes, which could conceivably result in a spuriously inflated difference between the two groups. However, the method of reporting was similar in both groups, and only faulty recollection may have biased the results. We believe that underreporting cannot fully account for the large difference in the rates of infection between the two groups. Furthermore, the significantly higher incidence of infection in the group with insulin-dependent diabetes in the postpartum period, a time when both groups are equally followed up, strengthens the conclusion that the pregnant woman with diabetes is indeed more prone to infection.

The risks for infection in this population may be related to the relative immune deficiencies induced by diabetes. Defective leukocyte chemotaxis,[2] depressed lymphocyte response to phytohemagglutinin,[3] impaired granulocyte adherence,[4] and impaired granulocyte phagocytosis[5, 6] have been implicated. Pregnancy itself is also thought to alter host susceptibility to infection. Although humoral immunity appears to be unaltered,[9] depression of selective aspects of cell-mediated immunity, such as depressed lymphocyte proliferation and alterations in monocyte/lymphocyte ratios have been reported.[7, 8, 10] Epidemiologic evaluation of infection in pregnancy is suggestive of a change in cell-mediated immunity.[13]

The diabetic gravid woman thus poses a special therapeutic challenge to the clinician. There are few studies

Table III. Type of infection in diabetic and control populations

	Diabetic	*Control*	*Significance*
Antepartum infection			
At least one infection (*n*, %)			
Urinary	8 (12%)	4 (6%)	$p < 0.05$
Genital	27 (42%)	5 (8%)	$p < 0.01$
Gastroenteritis	17 (26%)	3 (5%)	$p < 0.01$
Hepatitis	0	0	—
Respiratory	39 (60%)	7 (11%)	$p < 0.01$
Postpartum infection			
At least one infection (*n*, %)			
Wound infection and/or endometritis	5 (8%)	0 (0%)	$p < 0.05$

Table IV. Infection rate by organism in diabetic and control populations*

Organism	*Diabetic (n = 116)*	*Control (n = 28)*	*Significance*
Bacterial infection (*n*, %)	23 (20%)	11 (39%)	NS
Escherichia coli	5 (4%)	4 (14%)	
Streptococcus	4 (3%)	5 (18%)	
Bacteroides	0	0	
Chlamydia	1 (1%)	0	
Enterobacter	0	0	
Gonorrhea	1 (1%)	0	
Gardnerella	9 (8%)	1 (4%)	
Klebsiella	1 (1%)	0	
Proteus	0	0	
Staphylococcus	2 (2%)	1 (4%)	
Pseudomonas	0	0	
Treponema pallidum	0	0	
Protozoa (*Trichomonas*) (*n*, %)	3 (3%)	1 (4%)	NS
Viral infection (*n*, %)	57 (49%)	14 (50%)	NS
Herpes	2 (2%)	1 (4%)	
Cytomegalovirus	0	0	
Rubella	0	0	
Varicella	0	0	
Presumed viral syndrome	55 (47%)	13 (46%)	
Fungi (*Candida albicans*) (*n*, %)	33 (28%)	2 (7%)	0.01

*Whenever organism was recovered by culture or identified by direct examination (*Candida, Trichomonas,* nonspecific vaginitis).

Table V. Relation of prepartum infection to glycemic control in diabetic women*

	With prepartum infection (n)†	*Without prepartum infection (n)‡*	*Significance§*
Glycosylated hemoglobin (%)			
Trimester 1	9.69 ± 0.38	8.99 ± 0.47	NS
Trimester 2	8.06 ± 0.29	7.70 ± 0.40	NS
Trimester 3	7.65 ± 0.23	7.48 ± 0.17	NS
Mean preprandial glucose concentration (mg/dl)			
Trimester 1	132 ± 12	110 ± 21	NS
Trimester 2	113 ± 10	98 ± 13	NS
Trimester 3	130 ± 12	119 ± 16	NS
Mean postprandial glucose concentration (mg/dl)			
Trimester 1	154 ± 10	142 ± 18	NS
Trimester 2	148 ± 11	162 ± 21	NS
Trimester 3	143 ± 9	142 ± 15	NS

*Mean ± SEM.

†Wilcoxon rank-sum (one-tailed test).

‡For each period: trimester 1 $n = 24$; trimester 2 $n = 32$; trimester 3 $n = 24$.

§For all trimesters $n = 9$.

Table VI. Relation of postpartum infection and glycemic control in diabetic women*

	With postpartum infection	*Without postpartum infection*	*Significance†*
Glycosylated hemoglobin (%)			
Delivery	7.46 ± 0.31	7.62 ± 0.21	NS
Postpartum	8.48 ± 0.41	8.8 ± 0.32	NS
Mean preprandial glucose concentration (mg/dl)			
Delivery	123 ± 12	132 ± 9	NS
Postpartum	155 ± 19	160 ± 14	NS
Mean postprandial glucose concentration (mg/dl)			
Delivery	138 ± 15	147 ± 11	NS
Postpartum	113 ± 29	180 ± 34	NS

*Mean ± SEM.

†Wilcoxon rank-sum (one-tailed test).

of infection in diabetic pregnancies. In 1973 Vejlsgaards reported an increased incidence of urinary tract infection in diabetic gravid women compared with nondiabetic gravid women.[14] In a study restricted to postpartum infections, Diamond et al.[15] also observed an increased rate of postpartum wound infection, endometritis, or both in diabetic pregnant women. Our study confirmed these findings and evaluated a broader range of infectious agents and involved more organ systems. Our data show that diabetic gravid women are also more susceptible to gastrointestinal, respiratory, and wound infections when compared with a matched control population.

The association between glycemic control and infection is difficult to explore because infection may theoretically cause, or be caused by, poor glycemic control. Rayfield et al.[16] demonstrated a striking direct correlation between the overall prevalence of infection and the mean plasma glucose concentrations (representing three or more fasting glucose concentrations taken at times when no evidence of infection existed) in 241 nonpregnant diabetic women. Glycemic control was not evaluated in the studies by Vejlsgaards[14] and Diamond et al.[15] We attempted to relate glycemic control temporally to the incidence of infection of the women with insulin-dependent diabetes and found conflicting results. The differences in indices of glycemic control between the subgroup in whom infection developed and the subgroup in whom infection did not develop were not statistically significant. Further analysis showed that the mean hemoglobin A_1 obtained before the first infectious episode was significantly higher than

the mean hemoglobin A_1 of the same women obtained around the time of infection. Although the clinical significance of a small difference in hemoglobin A_1 concentrations may be doubtful, the theoretic implications of such a difference may be valuable. Because glycemic control is ordinarily hampered during infection and hemoglobin A_1 level was actually higher *before* the infection than *during* the infection, this may favor the thesis that poor glycemic control temporally precedes infection. Since hemoglobin A_1 gradually decreases as pregnancy progresses and glycemic control is achieved,[17] it is difficult to ascertain whether the statistically significant difference in hemoglobin A_1 before and during the infection can be attributed to this natural decline or to the relationship between glycemic control and infection. Thus the specific role of glycemic control in the aggravation of the risk of infection in pregnancy remains unanswered.

In conclusion, despite advances in current medical care, the diabetic pregnant woman is at significant risk for the development of infection involving most organ systems in both the antepartum and postpartum periods. Infection appears to be associated with poor glycemic control, but the temporal relationship is difficult to analyze. Whether further improvement in metabolic control will reduce the risk for infection in the pregnant woman with insulin-dependent diabetes is unclear at this time.

REFERENCES

1. Krall LP, ed. Joslin diabetes manual. 12th ed. Philadelphia: Lea & Febiger, 1989:267-8, 282, 291-4.
2. Mowat AG, Baum J. Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. N Engl J Med 1971;284:621-7.
3. MacCuish AC, Urbaniak SJ, Campbell CJ, Duncan L, Irvine WJ. Phytohemagglutinin transformation and circulating lymphocyte subpopulations in insulin-dependent diabetic patients. Diabetes 1974;23:708-12.
4. Bagdade JD, Stewart M, Walters E. Impaired granulocyte adherence: a reversible defect in host defense in patients with poorly controlled diabetes. Diabetes 1978;27:677-81.
5. Bagdade JD, Root RK, Bulger RJ. Impaired leukocyte function in patients with poorly controlled diabetes. Diabetes 1974;23:9-15.
6. Nolan CN, Beaty HN, Bagdade JD. Further characterization of the impaired bactericidal function of granulocytes in patients with poorly controlled diabetes. Diabetes 1978;27:889-94.
7. Weinberg ED. Pregnancy associated depression of cell mediated immunity. Rev Infect Dis 1984;6:814-31.
8. Falkoff R. Maternal immunologic changes in pregnancy: A critical appraisal. Clin Rev Allergy 1987;5:287-300.
9. Adelsberg BR. Immunology of pregnancy. Mt Sinai J Med (NY) 1985;52:5-10.
10. Sridama V, Pacini F, Yang SL, et al. Decreased levels of helper T cells: a possible cause of immunodeficiency in pregnancy. N Engl J Med 1982;307:352-6.
11. Lavin JP, Barden TP, Miodovnik M. Clinical experience with a screening program for gestational diabetes. AM J OBSTET GYNECOL 1981;141:491-4.
12. Kaplan LA, Cline D, Gartside P, Burstein S, Sperling M, Stein EA. Hemoglobin A_1 in hemolysates from healthy and insulin-dependent diabetic children, as determined with a temperature-controlled mini-column assay. Clin Chem 1982;28:138-41.
13. Brabin BJ. Epidemiology of infection in pregnancy. Rev Infect Dis 1985;7:579-603.
14. Vejlsgaards R. Studies on urinary infections in diabetes. Act Med Scand 1973;193:33-41.
15. Diamond MP, Entmann SS, Salyer SL, Vaughn WK, Boehm FH. Increased risk of endometritis and wound infection after cesarean section in insulin-dependent diabetic women. AM J OBSTET GYNECOL 1986;155:297-300.
16. Rayfield EJ, Ault MJ, Keusch GT, Brothers MJ, Nechemias C, Smith H. Infection and diabetes: the case for glucose control. Am J Med 1982;72:439-50.
17. Widness JA, Schwartz HC, Kahn CB, Oh W, Schwartz R. Glycohemoglobin in diabetic pregnancy: a sequential study. AM J OBSTET GYNECOL 1980;136:1024-30.

The effect of race on the relationship between fetal death and altered fetal growth

Richard Ferguson, MS,[a] and Stephen A. Myers, DO[b]
Chicago, Illinois

This population study examines racial differences in the relationship between birth weight and fetal death. An earlier report showed that a birth weight that results in a fourfold increased risk of stillbirth is not a constant birth weight percentile (2nd percentile at 25 weeks, 17th percentile at 42 weeks). This analysis was applied to 782,430 white and black births in Illinois from 1980 to 1984. Mean and 10th percentile growth for white and black infants is identical before 34 weeks' gestation and growth diverges by 250 gm at term, with white infants being larger. Race-specific birth weights resulting in quadrupling of the stillbirth rate were determined with an exponential regression analysis of the relationship between birth weight and fetal death rate for each gestational age. The data indicate (1) that the birth weights resulting in quadrupling the stillbirth rate are substantially above the Denver 10th percentile and the previously unpublished race-specific Illinois 10th percentiles and (2) that at term white infants demonstrate this constant risk at the 12th percentile, whereas black infants exhibit the risk at the 18th percentile. From these data we conclude that black fetuses are more sensitive than white fetuses to factors that adversely affect growth and that continued use of "race-neutral" data for clinical management in racially heterogenous populations will not accurately predict the risk of stillbirth. (AM J OBSTET GYNECOL 1990;163:1222-30.)

Key words: Race, growth, birth weight, fetal death

The clinical evaluation of altered fetal growth during pregnancy largely depends on identification of fetuses predicted to lie below some standard point for any given gestational age, usually below the 10th percentile for birth weight. After birth the diagnosis is either confirmed or refuted with a similar approach. In a previous analysis of >850,000 births in Illinois between 1980 and 1984, we found that the 10th percentile of birth weight does not result in a constant outcome at each gestational age.[1] Specifically, quadrupling of the fetal death rate occurs at the 2nd percentile of birth weight at 25 weeks compared with the 17th percentile at 40 weeks' gestation. Moreover, the birth weights at which this increased fetal death rate occurs differ considerably from the published Denver 10th percentile and, importantly, from the previously unpublished Illinois 10th percentile curve.[1]

The effect of race on the relationship between birth weight and gestational age has received some attention over the years in the medical literature.[2-8] However, it is still common practice to evaluate altered growth by using "race-neutral" criteria.[9] The purpose of this study is to examine the effect of race on the outcome-oriented risk assessment previously reported, as determined by the birth weight that results in the quadrupling of the stillbirth rate versus gestational age. If substantial differences in race-specific outcome data exist, the further utility of "race-neutral" data for clinical management would be questioned.

Material and methods

The state of Illinois computer data file of all births in Illinois between 1980 and 1984 contains data on 796,475 singleton births for which either birth or death certificate includes birth weight, gestational age, and white or black race. Working certificates are completed at the hospital of birth and are sent to the Illinois Department of Public Health where final certificates are prepared and the data entered into the computer data file. In Illinois 10% of the live births are to Hispanic mothers. However, until 1988 no separate identifier was present on the Illinois fetal death certificates. Therefore, separate Hispanic analysis could not be performed.[10] For white and black infants, we determined the number of fetal deaths at each gestational age along with race-specific mean and modal birth weights for

From the Division of Health Information and Evaluation, Biostatistics Section, Illinois Department of Public Health,[a] and the Division of Maternal-Fetal Medcine, Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center.[b]
Received for publication December 21, 1989; revised May 7, 1990; accepted June 2, 1990.
Reprint requests: Stephen A. Myers, DO, Department of Obstetrics and Gynecology, Chicago Medical School, Director of Maternal-Fetal Medicine, Mount Sinai Hospital Medical Center, Room F208, California Ave. at 15th St., Chicago, IL 60608.
6/1/22868

Table I. Gestational age and birth weight distribution, white births

Gestational age (wk)	*No. live-born (n = 620,088)*	*No. stillborn (n = 3,650)*	*Fetal death rate at mode (n/1000)*	*Mean birth weight (gm)*	*Modal birth weight (gm)*	*10th Percentile (gm)*
25	527	151	147.00	746	737	455
26	606	130	47.00	818	964	512
27	553	140	112.00	931	907	535
28	643	131	48.00	1136	1219	670
29	787	124	35.00	1276	1361	714
30	974	169	75.00	1484	1474	834
31	1366	142	28.00	1798	1814	1094
32	1855	166	19.00	1952	1899	1272
33	3150	182	11.00	2264	2240	1561
34	6540	193	9.0	2741	2381	1868
35	10927	178	5.9	2892	2637	2095
36	18541	253	4.7	3022	2835	2303
37	35303	268	1.6	3165	3062	2512
38	75115	293	0.9	3309	3175	2705
39	136825	331	0.7	3440	3402	2852
40	154880	300	0.6	3539	3629	2944
41	113180	258	0.7	3614	3629	3000
42	58316	241	1.5	3637	3629	2992

each gestational age using the univariate procedure of Statistical Analysis System (SAS Institute, Inc., Cary, N.C.). Obvious outliers (several newborns with birth weights >4000 gm at 25 weeks were found) were eliminated below 34 weeks by the following correction procedure. Because these outliers would affect only the mean, an assumption was made that the birth weight distribution at any given gestation was symmetric about the mode. We determined the number of 100 mg units for birth weights lower than the mode that contained at least 2% of the births for that gestational age. That number of 100 gm units was then counted off for birth weights higher than the mode. All births above that point were discarded. No correction of the data was performed beyond 34 weeks' gestational age. As a result a total of 8,637 live births and 63 stillbirths were deleted, leaving 787,775 deliveries for subsequent analysis. These corrected data were used to determine the race-specific mean birth weight and race-specific 10th percentile determinations for each gestational age (Table I).

For each race and within each gestational age group, the fetal death rate was determined for each birth weight increment of 100 gm. This was calculated by taking the number of fetal deaths divided by the total number of live births plus fetal deaths in births occurring in each 100 gm increment. Within each gestational age group, an exponential regression analysis of the relationship between birth weight and fetal death rate was made. For each gestational age, a nonlinear, inverse relationship was observed; as birth weight decreases, the fetal death rate increases in an exponential fashion. The fetal death rate at the modal birth weight was multiplied four times, and an estimate of the birth weight at which the modal fetal death rate quadrupled was determined (designated BW_{FDR4x}). This process has been reported previously in detail.[1] This procedure was repeated for each gestational age week within each racial group, and the results are tabulated both in grams and as race-specific birth weight percentile. Smoothed "curves" for mean, 10th percentile, and birth weight at which the modal fetal death rate quadrupled were constructed with use of third-degree polynomial regression analysis.

Results

The number of live births, fetal deaths, mean, and modal birth weights for each gestational age are tabulated for white and black births in Tables I and II, respectively. Table III contains the race-specific 10th percentile, and the birth weight resulting in quadrupling of the modal fetal death rate, expressed in grams and percentile for each race.

Comparison of white and black birth weight versus gestational age curves is represented in Fig. 1. As seen in the figure, the mean and 10th percentile curves are nearly identical before 34 weeks' gestation. After 34 weeks there is a gradual increase in mean and 10th percentile birth weights among whites of 250 gm by 40 weeks' gestational age.

The comparison of the race-specific 10th percentile of birth weight to the race-specific birth weight resulting in the quadrupling of the modal stillbirth rate are shown for white and black births in Figs. 2 and 3, respectively. As shown in Fig. 2, for white patients the birth weight resulting in a constant stillbirth rate is parallel to the 10th percentile, generally around the 12th percentile. In contrast, black infants do not achieve this

Table II. Gestational age and birth weight distribution, black births

Gestational age (wk)	*No. live-born (n = 162,342)*	*No. stillborn (n = 1695)*	*Fetal death rate at mode (n/1000)*	*Mean birth weight (gm)*	*Modal birth weight (gm)*	*10th Percentile (gm)*
25	481	107	53.00	739	794	443
26	512	89	80.00	901	964	461
27	487	77	85.00	998	1021	508
28	553	85	45.00	1140	1304	597
29	643	96	102.00	1296	1191	706
30	760	78	57.00	1473	1361	853
31	943	105	23.1	1682	1814	1009
32	1345	68	25.3	1954	1800	1272
33	2114	96	18.0	2203	2050	1518
34	3397	95	9.6	2437	2350	1782
35	6461	96	3.5	2802	2637	2048
36	9347	117	2.8	2892	2863	2192
37	14915	122	2.0	3005	2920	2369
38	24630	125	1.1	3118	3062	2514
39	33641	106	0.8	3214	3260	2615
40	30972	110	0.7	3287	3402	2674
41	20204	69	0.9	3330	3402	2695
42	10937	54	0.8	3327	3260	2688

Table III. Comparison of 10th percentile birth weight and birth weight resulting in quadrupling stillbirth rate for white and black births

	White births			*Black births*		
Gestational age (wk)	*10th Percentile (gm)*	BW_{FDR4x} *(gm)*	BW_{FDR4x} *as percentile*	*10th percentile (gm)*	BW_{FDR4x} *(gm)*	BW_{FDR4x} *as percentile*
25	455	227	1	443	507	26.0
26	512	606	18.5	461	330	5.0
27	535	460	6.0	508	440	7.0
28	670	824	19.5	597	718	15.5
29	714	917	20	706	224	1.0
30	834	854	11	853	764	7.0
31	1094	1310	18.5	1009	1302	23.0
32	1272	1448	17.5	1272	1141	6.0
33	1561	1836	22	1518	1510	9.5
34	1868	1956	12.5	1782	1815	11.0
35	2095	2133	11	2048	2191	15.0
36	2303	2330	11	2192	2376	17.5
37	2512	2626	14.5	2369	2463	14.0
38	2705	2764	18.5	2514	2626	15.0
39	2852	2930	13.0	2615	2715	14.0
40	2944	3062	15	2674	2896	20.5
41	3000	3063	12.5	2695	2850	16.5
42	2992	3020	11	2688	2850	18

BW_{FDR4x} Birth weight at which modal fetal death rate quadrupled.

magnitude of risk until reaching birth weight less than the 5th percentile in earlier gestation, whereas this risk is reached with infants in the 15th to 18th percentile at term (Fig. 3).

Comment

These population-based data support two important conclusions. First, race-specific mean and 10th percentile birth weight curves for white and black newborns do not differ substantially before 34 weeks' gestation and diverge by approximately 250 gm at term. Second, the race-specific birth weight that results in quadrupling of the fetal death rate, expressed in either grams or as race-specific birth weight percentile, is not the same for white and black patients. Because both race-specific risk curves are above the race-specific 10th percentiles, and substantially above the Denver 10th percentile, continued widespread use of the Denver data must be questioned (Fig. 4, shaded areas). Fetuses or newborn within the shaded area are not currently considered either as having growth retardation or as being at substantial risk. However, as these data indicate, fetuses within this area have greater than a fourfold increased risk of stillbirth compared with their more nor-

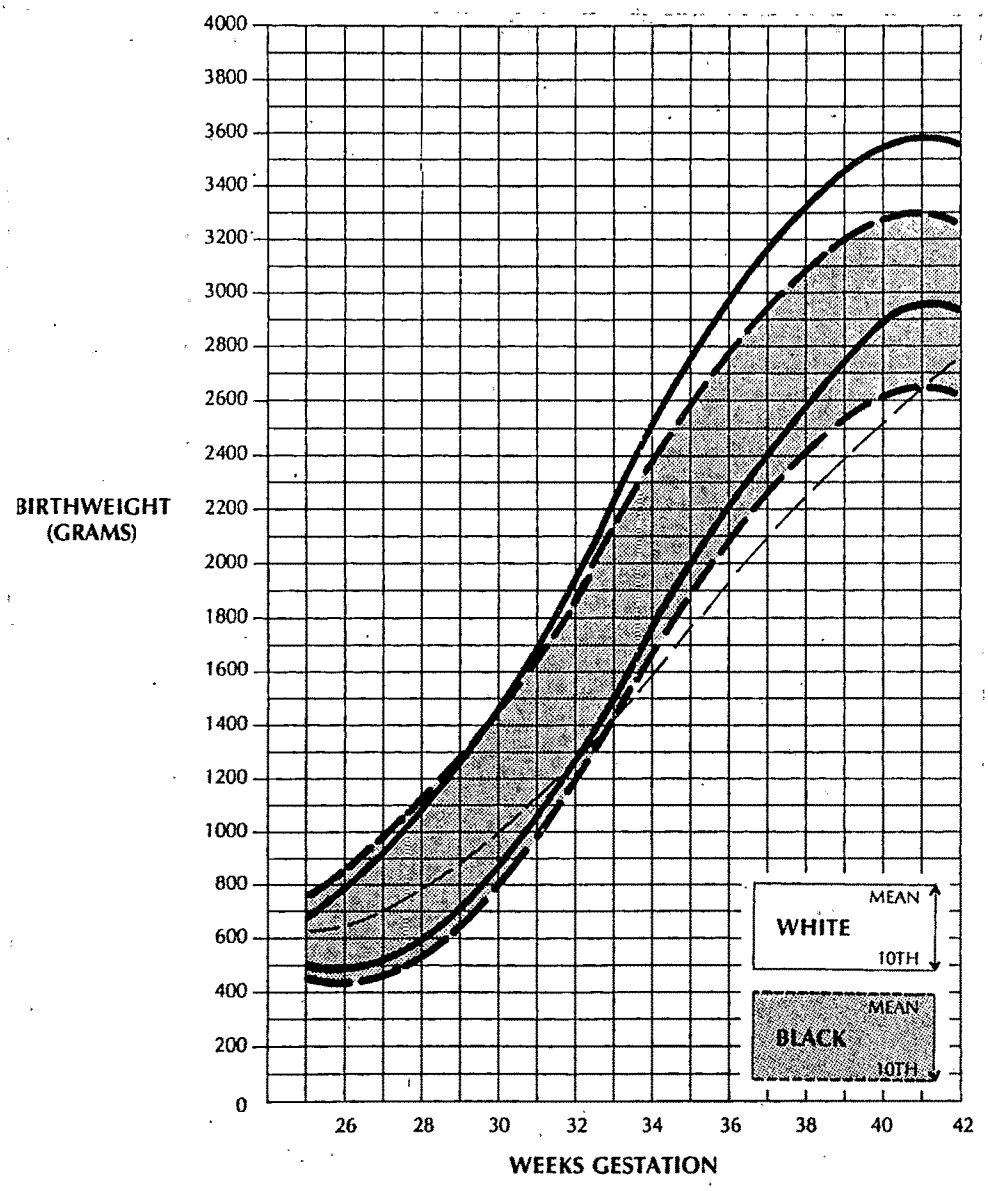


Fig. 1. Smoothed growth "curves" of mean and 10th percentile for white and black patients. *Thin lower line* represents the Denver 10th percentile.

mally grown (modal birth weight) peers. As a result significant underestimations of risk occurs for this group. This is precisely the period in gestation when the majority of nongenetic factors associated with altered growth exert their effect. Before this period the Denver data tend to overestimate risk, especially for black infants. However, since clinical recognition of intrauterine growth retardation at this stage is uncommon, this overestimation has not had much relevance. Moreover, the small sample size of Denver data at this "end of the curve" raises the possibility of statistical inaccuracy related to curve fitting accounting for much of the discrepancy, compared with the larger numbers reported from Illinois.

Relative risks calculated with both the Denver data and the race-specific data are listed in Table IV. Comparison of these risk multiples demonstrates the magnitude of underestimation that would result from continued use of those data. As seen in Table IV, for white births the use of the 10th percentile would predict an average 4.9-fold risk of stillbirth at the modal birth weight beyond 33 weeks' gestation. By comparison, use of the 10th percentile for black births would result in an average of 6.1-fold risk of stillbirth rate for the same period of pregnancy. Importantly, use of the Denver 10th percentile results in an unacceptably high relative risk of still birth: from 11 to 22 times the modal fetal death rate for white births (from 34 to 40 weeks' gestation, see table). Similarly, a somewhat smaller but comparably increased risk would result from use of the Denver 10th percentile to predict risk of stillbirth for blacks.

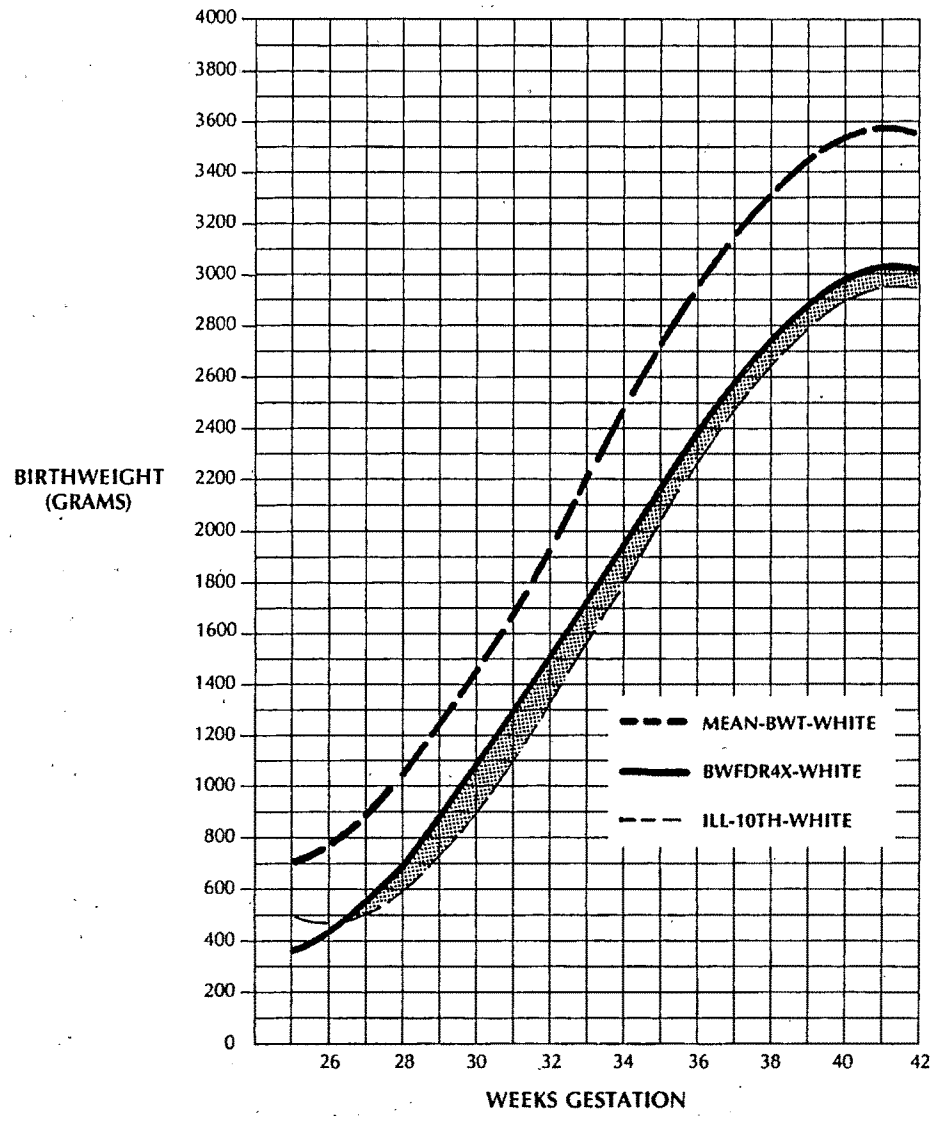


Fig. 2. Relationship between birth weight predicted to result in quadrupling of fetal death rate of each gestational age compared with 10th percentile for white patients. *Shaded area* represents birth weights underestimated to be at risk if race-specific 10th percentile were used.

There have been previous attempts to compare birth weights of white and black newborns.[2, 3, 5-9] Although some of these studies suffer from either small total samples or large data sets only after 28 to 30 weeks, the results vary little from these data. The study of Brenner et al.[8] has been consistently used as a "standard" in the United States. Although percentiles for only the pooled data (race-neutral) are recorded, race-specific mean birth weights for each race are given, but only in a figure inset and only beyond 36 weeks. In Fig. 3 of their study, a 225 gm mean birth weight difference between white and black infants is shown at 40 weeks' gestation. This does not differ much from the 250 gm difference we report at 40 weeks' gestation (Tables I and II). Unfortunately, there is no race-specific 10th percentile data in the Brenner paper. We speculate that if this had been calculated, percentiles similar to our own data would have been generated. Consequently, there is little reason to suspect that significant geographic differences exist for white and black patients. Any race-specific growth curve would be as useful in New York or California as in Chicago. Unlike the Brenner data, our population-based data includes race-specific mean and 10th percentile of birth weight for each gestational age between 25 and 42 weeks.

The relationship between percentile growth and risk has not been precisely defined in the medical literature. Problems with standards for diagnosis of growth re-

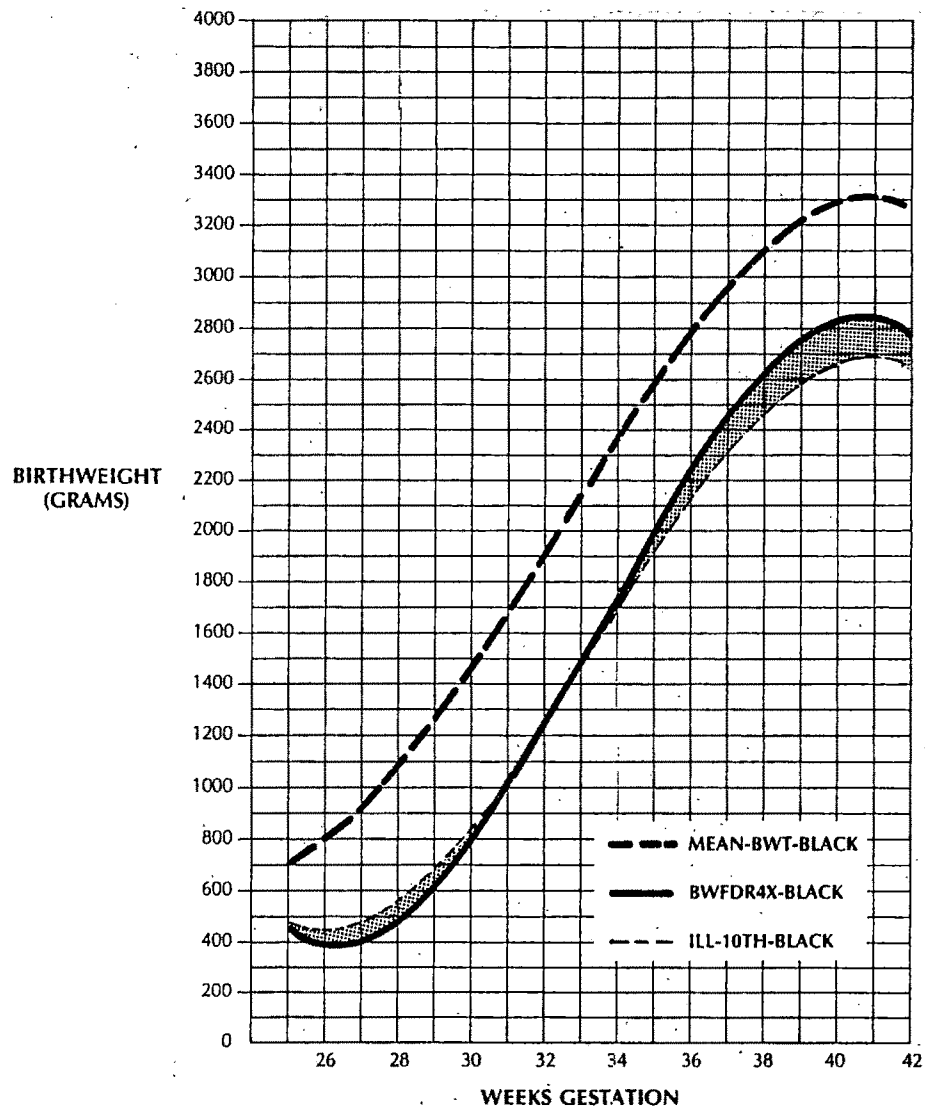


Fig. 3. Relationship between birth weight predicted to result in quadrupling of fetal death rate of each gestational age compared with 10th percentile for black patients. *Shaded area* represents birth weights underestimated to be at risk if race-specific 10th percentile were used.

tardation have recently been described by Goldenberg et al.[11] They recommended use of a national standard based on growth, at least partially recognizing difficulties related to heterogenous populations. However, they did not evaluate the use of an outcome-oriented risk assessment on determining the "standard," as discussed in our earlier analysis of this relationship. We observed that at each gestational age, the risk of stillbirth increases exponentially as birth weight decreases.[1] Unless the exponential nature of the relationship is appreciated, a tendency to underestimate risk occurs as birth weight becomes progressively smaller. Moreover, by determining an outcome-oriented criteria for assessing risk (the birth weight that results in quadrupling the stillbirth rate), we observed that this risk is not the same birth weight percentile from 25 to 42 weeks' gestation.[1] When this was observed in our earlier work, the analysis was applied with use of race-specific data to determine what racial difference in response to factors causing altered growth may exist.

When the data from the original analysis are compared with these data, it can be shown that given a population containing 20% blacks (as in Illinois), the addition of risk curves for whites and blacks would result in the curve originally reported.[1] It also follows, that as the racial make-up of any given population varies, so too would any single risk curve, and thus the 10th percentile. The continued reliance on race-

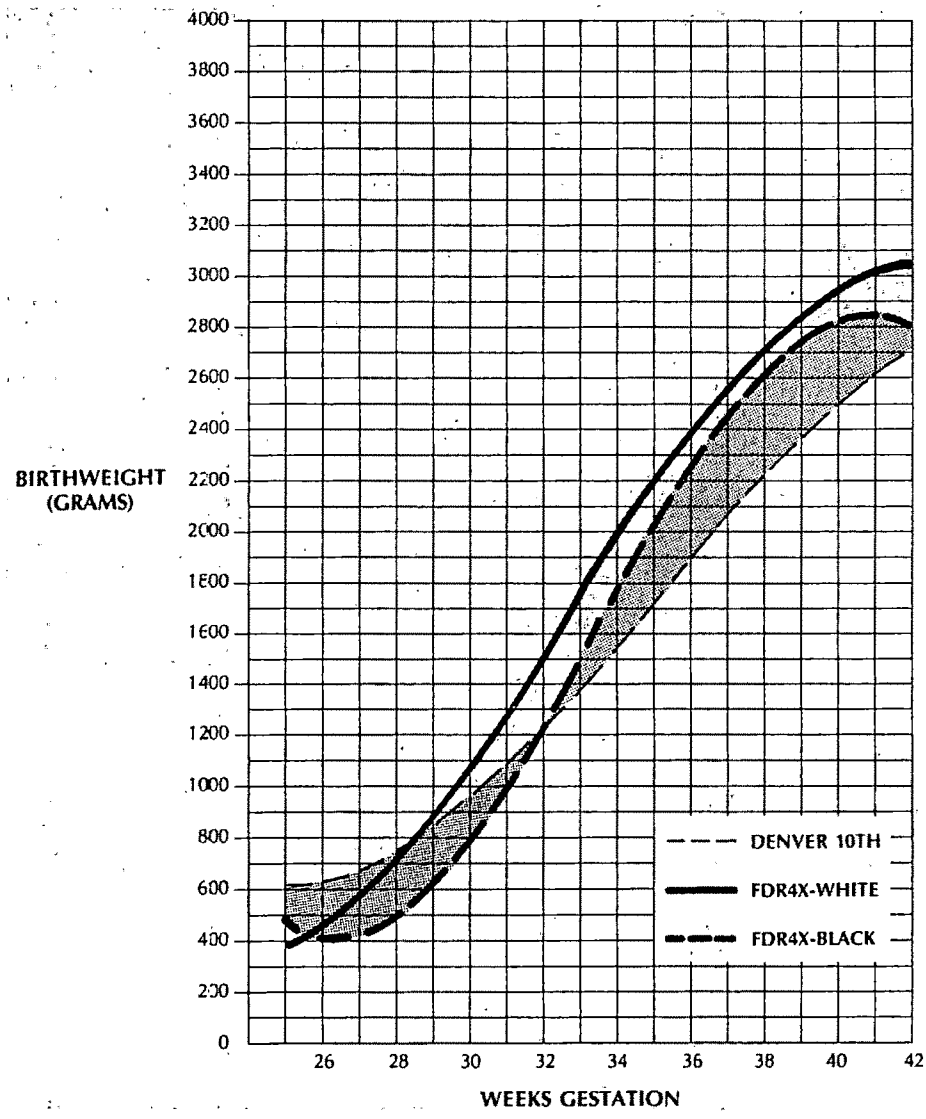


Fig. 4. Comparison of smoothed race-specific risk curves compared with Denver 10th percentile. *Heavier shaded area* represents birth weights underestimated at risk for blacks compared with Denver 10th percentile. *Lighter shaded area* represents comparable weights for white infants.

neutral, "homogenous" data sets for clinical assessments about growth, and therefore risk should be replaced. Data that is race-specific will surely result in more accurate determinations. As seen in Fig. 2, the risk curve for white patients consistently runs parallel and just above the 10th percentile, approximately the 12th percentile. In contrast, black infants demonstrate the same risk at progressively higher race-specific birth weight percentiles after 34 weeks' gestation, reaching the 18th percentile at 40 weeks. By comparison of the two race-specific birth weight percentiles resulting in the same adverse outcome, inferences about racial differences to the response to factors causing dimished fetal growth can be made. Specifically, whereas a white infant must "reach" the 12th percentile at term to have a quadrupled risk of stillbirth, a black infant has the same risk at a *larger* comparative birth weight for its race, the 18th percentile. If the exponential nature of the relationship between birth weight and fetal death is considered, it can be seen that if the black infant were to reach the 12th percentile, a five to six times increased risk of stillbirth would occur. After 34 weeks' gestation, black fetuses are more sensitive than white fetuses to factors causing altered fetal growth. The increased sensitivity results in an increased risk of stillbirth. Unless this increased sensitivity of blacks compared with whites is appreciated, further underestimations of risk for this group can occur. Similarly, although white fetuses may be relatively more resistant to altered growth, a difference of as much as 400 gm separates the white risk

Table IV. Relative risk of fetal death for white and black births

Weeks' gestation	White births		Black births	
	Multiple of modal FDR with Illinois 10th percentile for white births	*Multiple of modal FDR with Denver 10th percentile*	*Multiple of modal FDR with Illinois 10th percentile for black births*	*Multiple of modal FDR with Denver 10th percentile*
25	2.2	1.5	5.5	2.6
26	5.8	3.4	3.0	2.0
27	3.2	1.7	3.4	2.0
28	6.9	4.7	5.3	3.5
29	7.5	4.6	2.0	1.6
30	4.2	2.9	3.3	2.3
31	7.3	6.7	8.8	6.5
32	6.8	7.3	3.0	3.2
33	10.0	17.8	3.9	5.3
34	5.3	13.8	4.4	7.4
35	4.4	11.5	6.3	16.0
36	4.3	13.0	6.8	16.0
37	5.8	21.3	5.3	12.0
38	4.9	22.6	5.7	13.0
39	5.0	17.6	5.1	8.3
40	5.3	13.2	7.3	9.6
41	4.6	11.0	5.9	6.6
42	4.3	7.8	8.0	7.0

FDR, Fetal death rate.

curve from the Denver tenth percentile. As a result significant underestimation of risk for white fetuses results as long as use of the Denver 10th percentile is continued.

From a teleologic perspective, there is much to be said for the notion that, given a single gestational age group with thousands of birth weights, there is something very "normal" about the most common (modal) weight. It would appear that, given the variations in population, the modal weight is likely the birth weight "intended" for that gestational age. If the modal birth weight could be considered the most normal, we reasoned that the stillbirth rate at that weight therefore could be considered the "background" stillbirth rate for any given gestational age. The use of a factor of four was arbitrary. However, whereas doubling the stillbirth rate from 1.4 per 1000 to 2.8 per 1000 (using the 40-week gestational age group, for example)[1] could arguably be viewed as not necessarily a real increase, we reasoned that by the time a stillbirth rate quadruples, it clearly represents a negative biologic effect. Although smaller multiples may legitimately be considered as representing significant risk, the effect on our analysis should be noted. For example, whereas quadrupling the risk for blacks occurs at the 17th percentile at term, doubling the risk would occur above the 25th percentile. Although surveillance for this extremely large group may become realistically feasible in the future, we believe it impractical to provide reasonable fetal surveillance to the group based on current capabilities.

Fig. 4 shows a compilation of our data that is useful for patient management. It incorporates not only the racial differences in birth weights but also the racial differences in sensitivity to altered growth. Most importantly, the *actual* birth weight resulting in quadrupling the stillbirth rate can be determined. At 40 weeks' gestation, a white infant weighing <2950 gm (6 pounds, 8 ounces), or a black infant weighing <2800 gm (6 pounds 3 ounces) has greater than four times the risk of stillbirth compared with their more appropriately grown peers. These weights are substantially *above* weights generally perceived as small by the obstetric or pediatric providers. When compared with the Denver 10th percentile (as shown in Fig. 4), these "risk weights" average 400 to 500 gm *above* the weight generally perceived to represent risk. If these race-specific risk curves replace currently used data sets, the potential for identifying a significant portion of fetuses at risk for stillbirth will be enhanced.

REFERENCES

1. Myers SA, Ferguson R. A population based study of the relationship between altered fetal growth and fetal death. Obstet Gynecol 1989;74:325-31.
2. Freeman MC, Graves WL, Thompson RL. Indigent negro and caucasian birth weight-gestational age tables. Pediatrics 1970;46:9-15.
3. Williams RL. Intrauterine growth curves: intra- and international comparisons with different ethnic groups in California. Prev Med 1975;4:163-72.
4. Miller HC. Intrauterine growth retardation. Am J Dis Child 1981;135:944-8.
5. Eberstein IW, Parker JR. Racial differences in infant mortality by cause of death: the impact of birth weight and maternal age. Demography 1984;21:309-21.

6. Johar R, Rayburn W, Weir D, Eggert L. Birth weights in term infants: a 50-year perspective. J Reprod Med 1988;33:813-6.
7. Creasy RK, Resnik R. Intrauterine growth retardation. In: Creasy RK, Resnik R, eds. Maternal fetal medicine. Philadelphia: WB Saunders, 1984:493.
8. Brenner WP, Edelman DA, Hendricks GH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555-64.
9. Sweet A. Classification of the low birthweight infant In: Klaus MH, Fanaroff AA, eds. Care of the high risk neonate, Philadelphia: WB Saunders, 1986:69-95.
10. Ferguson R, Puente S, Ellinger J, Noak J. Proceedings of 1989 Public Health Conference on Records and Statistics. Washington, DC: US Public Health Service, July 1989: 492-7; PHS 90-1214.
11. Goldenberg RL, Cutter GR, Hoffman HJ, Foster JM, Nelson KG, Hauth JC. Intrauterine growth retardation: standards for diagnosis. AM J OBSTET GYNECOL 1989;161: 271-7.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Effects of intravascular fetal blood transfusion on fetal intracardiac Doppler velocity waveforms

Giuseppe Rizzo, MD,[a] Kypros H. Nicolaides, MD,[b] Domenico Arduini, MD,[a] and Stuart Campbell, MD[b]

Rome, Italy, and London, England

In 12 fetuses from pregnancies with red blood cell isoimmunization Doppler velocity waveforms were recorded at the level of atrioventricular valves immediately before and at 15-minute intervals for 2 hours after the intravascular transfusion. The left and right cardiac outputs, the ratio between the peak velocities during early passive ventricular filling and active atrial filling at the level of both ventricles as well as the heart rate were calculated. Before transfusion, the left and right cardiac outputs were significantly higher than reference ranges for gestation that were constructed from the cross-sectional study of 187 normal pregnancies. After transfusion there was a significant temporary fall in right and left outputs associated with increased ratios between the peak velocities during early passive ventricular filling and active atrial filling. Within 2 hours after transfusion both parameters returned toward the normal range. In addition, no significant changes were found for fetal heart rate values before and after transfusion. The fall of cardiac output was significantly related to the amount of expansion of the feto-placental volume. (Am J Obstet Gynecol 1990;163:1231-8.)

Key words: Red blood cell isoimmunization, intrauterine intravascular transfusion, cordocentesis, fetal echocardiography, Doppler ultrasonography

Intravascular fetal blood transfusion by cordocentesis represents a significant improvement in the treatment of red blood cell isoimmunization.[1] However, to limit the risks of repeated procedures and to shorten the duration of the transfusion the amount of blood transfused is usually high with respect to the estimated fetoplacental blood volume and is injected rapidly.

Up to now few data are available on the effects of such treatment on fetal hemodynamics.[3, 4] The advent of Doppler echocardiography has made it possible to study intracardiac blood flow velocity waveforms in the human fetus and to obtain reliable assessment of cardiac output.[5, 6] We therefore examined the effect of intravascular blood transfusion on cardiac hemodynamics in a group of fetuses from pregnancies with red blood cell isoimmunization.

Material and methods

Twelve pregnancies complicated by red blood cell isoimmunization undergoing intravascular transfusion were considered for this study. All pregnancies were singleton and accurately dated by certain last menstrual period and early ultrasonographic examination. All the fetuses had been previously transfused and the time interval from the preceding transfusion ranged between 2 and 4 weeks. The criteria followed for the timing of transfusions has been reported in detail elsewhere.[1] Although none of the fetuses had ultrasonographic evidences of hydrops, in all the cases the fetal hemoglobin concentration was below the second SD (mean difference, −3.62 SD; range, −2.43 to −5.16 SD) of our reference range for gestation.[7]

The mean (± SD) gestational age at the time of the study was 31.42 ± 3.18 weeks (range, 26 to 37). Before transfusion all patients gave their informed consent to participate in the study.

Doppler recordings. Commercially available Acuson 128 color Doppler equipment (Acuson, Montain View, Calif.) with 3.5 or 5 MHz sector probes was used for all measurements. Recordings were performed with the mother lying in a comfortable semirecumbent position. All the recordings were performed by one operator (G. R.) and the technique used has been reported in detail.[8] Briefly, an apical four-chamber view of the fetal heart was first obtained. The color flow mapping function was then superimposed and the transmitral and transtricuspid flow observed. The sample volume was placed immediately below either the mitral or tricuspid valves in the point of maximum flow velocity, as ex-

From the Laboratory of Fetal Physiology, Department of Obstetrics and Gynecology, Università Cattolica S. Cuore,[a] and Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics and Gynecology, King's College Hospital.[b]

Supported in part by the Italian National Council of Research (CNR grant No. 89.03875.CT04.

Received for publication December 22, 1989; revised June 4, 1990; accepted June 6, 1990.

Reprint requests: Dr. G. Rizzo, Ist Cl. Ostetrica e Ginecologica, Università Cattolica S. Cuore, Largo A. Gemelli, 8, Roma, Italy 00168.

6/1/22990

Table I. Hematologic values measured before and after intravascular transfusion

Case no.	*Gestation (wk)*	*Pretransfusion hemoglobin (gm/dl)*	*Pretransfusion hematocrit (%)*	*Posttransfusion hemoglobin (gm/dl)*
1	30	10.3	31.1	16.2
2	33	9.9	29.1	17.3
3	32	10.4	30.3	17.0
4	35	12.0	34.7	16.1
5	26	8.5	24.9	15.2
6	31	9.6	28.7	17.1
7	31	10.4	30.3	15.0
8	37	12.5	39.4	15.9
9	32	10.4	31.4	15.8
10	27	8.9	26.5	15.3
11	34	9.2	27.8	16.9
12	29	10.9	31.3	14.8
Mean	31.42	10.25	30.46	16.05
SD	3.18	1.17	3.80	0.87

Table II. Left and right cardiac output in healthy fetuses (control) and in anemic fetuses before and 2 hours after intravascular transfusion

	Control	*Pretransfusion*	*Posttransfusion*
LVO (ml/min/kg)	249.12 ± 94.23	364.57 ± 121.18	300.73 ± 98.36
RVO (ml/min/kg)	295.45 ± 97.41	398.76 ± 111.44	346.41 ± 94.35

LCO, Left cardiac output; *RCO,* right cardiac output.

Control versus pretransfusion, $p \leq 0.01$ (the unpaired Student t test). Pretransfusion versus posttransfusion, $p \leq 0.01$ (the paired Student t test).

pressed by the color brightness, and velocity waveforms were then recorded. Doppler and two-dimensional images were recorded on standard ½-inch videotape for subsequent analysis. Recordings were performed immediately before (0) and at 15-minute intervals for 2 hours after the intravascular transfusion (+15, +30, +45, +60, +75, +90; +105, +120). Recordings obtained with an angle of insonation >30° or during periods of fetal body and breathing movements were rejected.

Atrioventricular valve diameters were evaluated from the two-dimensional images obtained from optimal apical four-chamber views. Measurements were performed in playback on video-recorded images frozen during diastole when showing the maximized diameters. Measurements were repeated on 10 different cycles for each valve and the values obtained were averaged. Repeated diameter measurements varied <0.6 mm for either valve. Valvular area was calculated assuming a circular cross-section.

Permanent records of all the velocity waveforms were obtained from the videotape by means of a strip chart recorder. The printouts were labeled with random numbers. The observer involved in the later analysis was not informed of the sequence of the printouts. Ten consecutive velocity waveforms were selected for each time interval from both atrioventricular valves and measurements of the following variables were made by use of the digitizing tablet of a computer (Cardio 800, Kontron, Oxford, England): (1) peak velocities during early ventricular filling (E wave) and atrial contraction (A wave); (2) mean temporal velocity; (3) fetal heart rate.

For each time interval a ratio between E peak and A peak velocities (E/A ratio) at the level of both atrioventricular valves was calculated. Absolute right and left cardiac outputs (milliliters per minute) were derived by multiplying the tricuspid or mitral mean temporal velocities, valvular area, and heart rate. The ratio between right and left cardiac output was then calculated. Cardiac output was also expressed as milliliters per minute per kilogram. The fetal weight was estimated from the ultrasonographic measurements of fetal biparietal diameter and abdominal circumference[9]; in all the cases abdominal circumference values ranged between the 10th and 90th percentile of our reference limits.

The data of the fetuses with red blood cell isoimmunization were compared with reference ranges constructed on the cross-sectional study of 187 fetuses from normal pregnancies between 20 and 40 weeks' gestation.

Posttransfusion hematocrit (%)	*Blood volume transfused (ml)*	*Transfusion rate (ml/min)*	*Expansion (%)*	*Expansion rate (%/min)*
48.5	80	14	63.74	11.15
51.5	150	7.9	93.72	4.94
44.1	100	20	61.06	12.21
46.4	110	7.3	36.34	2.41
43.9	80	5	99.48	6.22
50.4	120	15	106.37	13.30
44.1	100	8.3	61.06	5.07
50.9	160	9.4	79.19	4.65
47.8	70	5.8	49.55	4.11
45.6	60	15	65.86	16.47
50.9	100	12	62.60	7.51
46.4	70	16	48.64	12.07
47.54	100.00	11.30	69.87	8.34
2.87	31.33	4.7	21.54	4.49

In our laboratory the intraobserver coefficient of variation for all the measurements considered is <12%.

Method of transfusion. Intravascular fetal blood transfusion was performed in accordance with a previously described technique without maternal sedation or fetal paralysis.[1] The site and direction of the umbilical cord at its placental insertion was defined by real-time ultrasonographic scanning and a 20-gauge needle was inserted in the umbilical vein. A 2 ml sample of fetal blood was aspirated for measurement of hemoglobin (Coulter S Plus counter, Coulter Electronics Ltd., Luton, England) and blood gases (ABL 30 blood gas analyser, Radiometer, Copenhagen, Denmark). Fetal transfusion was performed with packed red blood cells compatible with the mother (mean hematocrit, 73.43%; SD, 7.18%; range, 63.0% to 87.8%). At the end of the transfusion the needle was flushed with normal 0.5 ml of saline solution and after 1 minute a sample of fetal blood was aspirated. The first 0.5 ml were discarded and the posttransfusion hemoglobin and hematocrit were subsequently evaluated.

The fetoplacental volume was determined as previously reported[2] and the percentage of its expansion after the transfusion was calculated: % Expansion = (Blood volume transfused/fetoplacental blood volume) × 100. Furthermore, we evaluated the expansion rate: (Expansion rate = Transfusion rate/fetoplacental blood volume) × 100.

Statistical analysis. The changes of E/A ratios, left cardiac output, right cardiac output, and heart rate were evaluated as the percentage change from the values obtained before transfusion (basal values). Data obtained at each time interval were averaged and reported as mean values ± 1 SD. Statistical analysis was performed with the use of analysis of variance for repeated measurements and the paired and unpaired student *t* test as indicated in the text. Relationships between the variables considered were tested by stepwise regression analysis. These analyses were performed on a Macintosh II computer with the Stat View II statistical package (Abacus Concepts Inc., Berkeley, Calif.).

Results

Intravascular transfusion was successful in all the fetuses studied and the individual hematologic values measured before and after transfusion are shown in Table I. Blood gas analysis performed before transfusion revealed P_{O_2}, P_{CO_2}, and pH values within 95% confidence interval of our reference ranges for gestation.[11]

Reliable recordings of right cardiac output and left cardiac output were obtained respectively 93.5% and 90.7% of the cases.

The prefusion values of left cardiac output, right cardiac output, and ratio of right cardiac output to left cardiac output of the fetuses with red blood cell isoimmunization are plotted on our reference ranges for gestation (mean and 95% confidence intervals) in Fig. 1. After correction for fetal weight the right cardiac output and left cardiac output were significantly higher (the unpaired Student *t* test, $p \leq 0.01$) than in the control group (Table II). Furthermore there was a tendency for the E/A ratios of both atrioventricular valves to be higher than those in our normal controls (Fig. 2) but this did not reach statistical significance.

After transfusion the right cardiac output and left cardiac output fell. Subsequently there was an increase in both parameters but to less than pretransfusion values (Fig. 3). Analysis of variance demonstrated the significance of these changes for both right cardiac output [$F(10,100) = 16.13, p \leq 0.001$] and left cardiac output [$F(10,97) = 13.47, p \leq 0.001$]. Significant differences

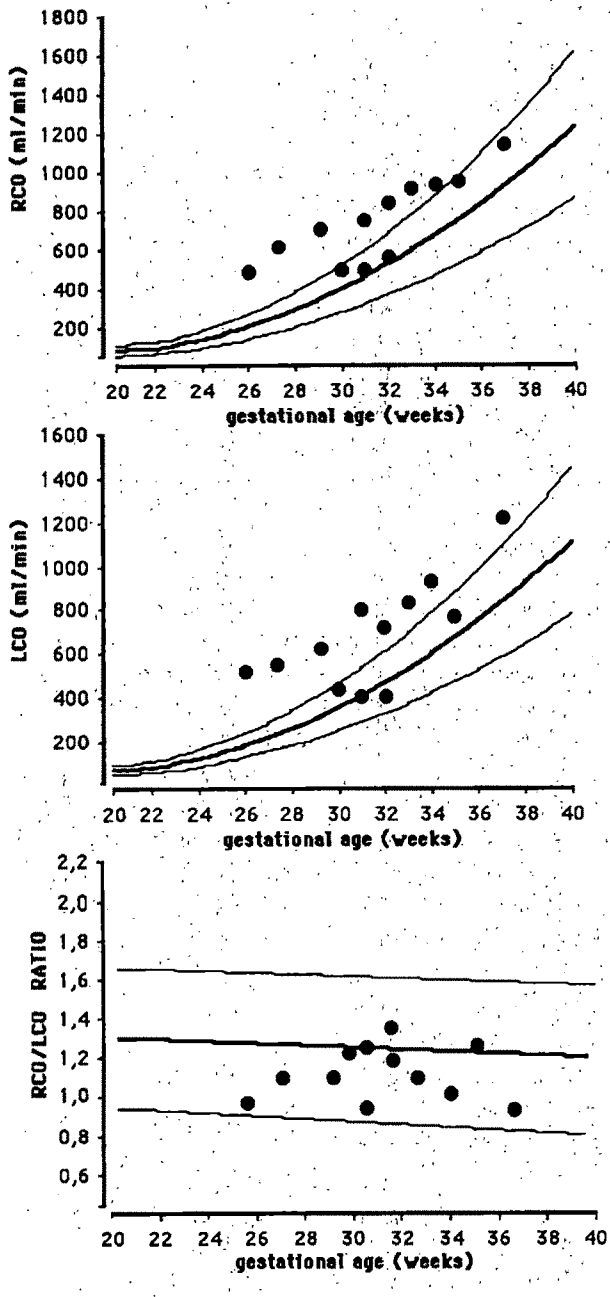


Fig. 1. Right cardiac output *(RCO) (top)*, left cardiac output *(LCO) (middle)*, and *RCO/LCO (bottom)* values obtained before transfusion plotted on our reference ranges for gestation (mean and 95% confidence intervals).

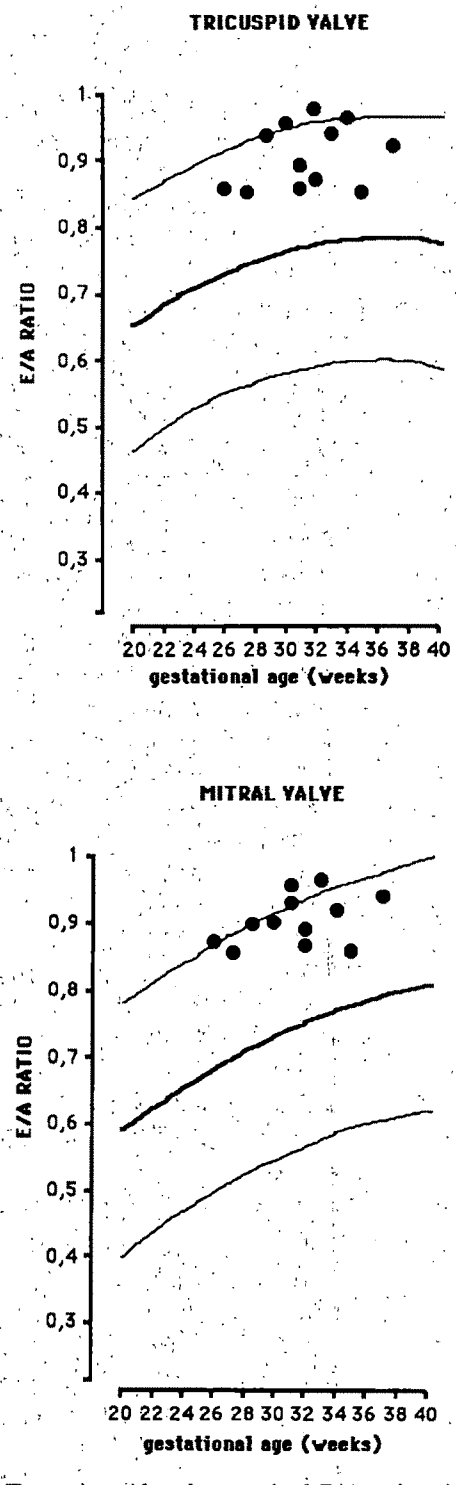


Fig. 2. Transtricuspid and transmitral E/A ratio values obtained before transfusion plotted on our reference ranges for gestation (mean and 95% confidence intervals).

were found (the paired Student t test) between pretransfusion values of right cardiac output and left cardiac output and +15, +30, +45, +60, +105, +120 values. Furthermore the E/A ratios were significantly modified after transfusion [tricuspid valve $F(10,100) = 8.21$, $p \leq 0.001$; mitral valve $F(10,97) = 4.59$, $p \leq 0.01$]. The highest value of E/A ratio were found at +15 minutes and then progressively returned toward basal values (Fig. 4).

No significant changes were found after transfusion for the ratio of right cardiac output to left cardiac output [$F(10,96) = 0.98$, NS] or fetal heart rate [$F(10,100) = 1.42$, NS]. The individual data obtained before and at the +15-, +30-, and +120-minute in-

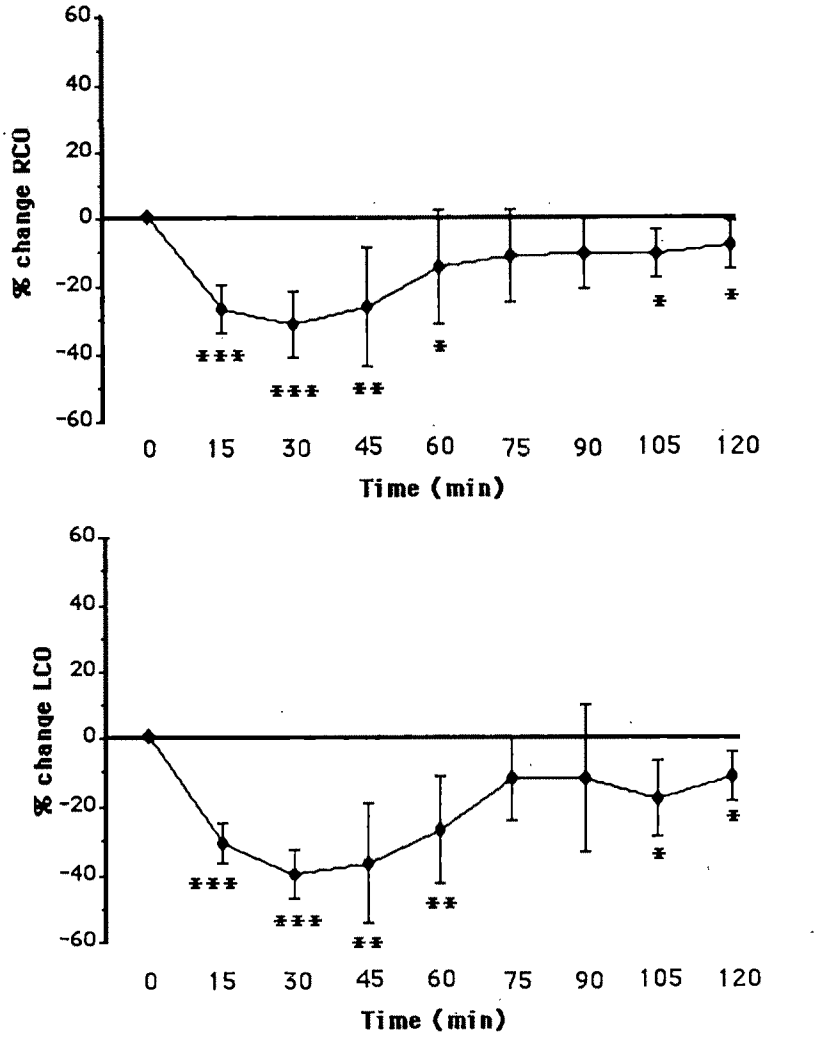


Fig. 3. Mean percentage changes (± 1 SD) of right cardiac output *(RCO)* and left cardiac output *(LCO)* after transfusion. Differences on comparison with pretransfusion values were evaluated by means of the paired Student *t* test (* = $p \leq 0.01$; ** = $p \leq 0.002$; *** = $p \leq 0.001$).

tervals after intravascular transfusion are provided in Table III.

Stepwise regression analysis revealed a significant linear correlation between the percentage of expansion of the fetoplacental blood volume (independent variable) and the maximum individual percentage fall of combined cardiac output (left cardiac output + right cardiac output) after transfusion (dependent variable); $r = 0.77$, $p \leq 0.01$, slope = 0.22, intercept, −21.01. There were significant correlations between the other Doppler parameters measured and either the volume of blood transfused or the percentage of expansion or the rate of expansion of the fetoplacental blood volume.

Comment

Our findings of increased fetal cardiac output in pregnancies with red blood cell isoimmunization is in agreement with previous reports on both the animal and the human fetus.[11-13] Although the mechanisms that cause the increase of cardiac output are still unclear two main factors have been suggested in animals[11]: first, decreased blood viscosity leading to increased venous return and cardiac preload, and second, peripheral vasodilatation as a result of a fall in blood oxygen content and therefore reduced cardiac afterload.

Previous studies in red blood cell isoimmunization have shown an increase of blood flow velocities from different fetal peripheral vessels including umbilical vein,[14] descending aorta,[3, 4, 15] and common carotid artery,[4] and the increase of velocity was related to the severity of anemia. The high pretransfusion E/A ratios found in this investigation suggest that cardiac preload is increased.[13] Furthermore, because the right cardiac output to left cardiac output ratio was normal there is no evidence for a redistribution of cardiac output sim-

Table III. Doppler echocardiographic measurements before and 15, 30, and 120 minutes after intravascular transfusion

Case no.	*Pretransfusion*						*Posttransfusion (+15 min)*					
	Tricuspid valve			*Mitral valve*			*Tricuspid valve*			*Mitral valve*		
	RCO	*HR*	*E/A*	*LCO*	*HR*	*E/A*	*RCO*	*HR*	*E/A*	*LCO*	*HR*	*E/A*
1	507	142	0.95	411	145	0.91	446	147	1.07	306	150	1.06
2	911	143	0.94	833	143	0.96	694	132	1.29	528	143	1.34
3	844	128	0.98	718	133	0.89	595	143	1.22	507	143	1.10
4	950	141	0.85	763	143	0.86	683	143	1.20	548	146	1.18
5	490	136	0.86	512	146	0.87	307	143	1.20	308	143	1.16
6	751	136	0.89	801	143	0.96	478	120	1.00	456	128	1.10
7	495	136	0.86	394	133	0.93	385	154	1.15	281	150	1.24
8	1136	130	0.92	1222	148	0.94	—	—	—	—	—	—
9	549	135	0.87	407	140	0.87	388	136	1.11	314	140	1.08
10	604	148	0.85	538	145	0.86	457	148	1.13	370	145	1.04
11	925	139	0.96	890	148	0.92	716	141	0.94	651	146	0.86
12	689	136	0.93	614	132	0.89	481	136	1.11	421	132	1.08

LCO, Left cardiac output; *RCO*, right cardiac output; *HR*, heart rate.

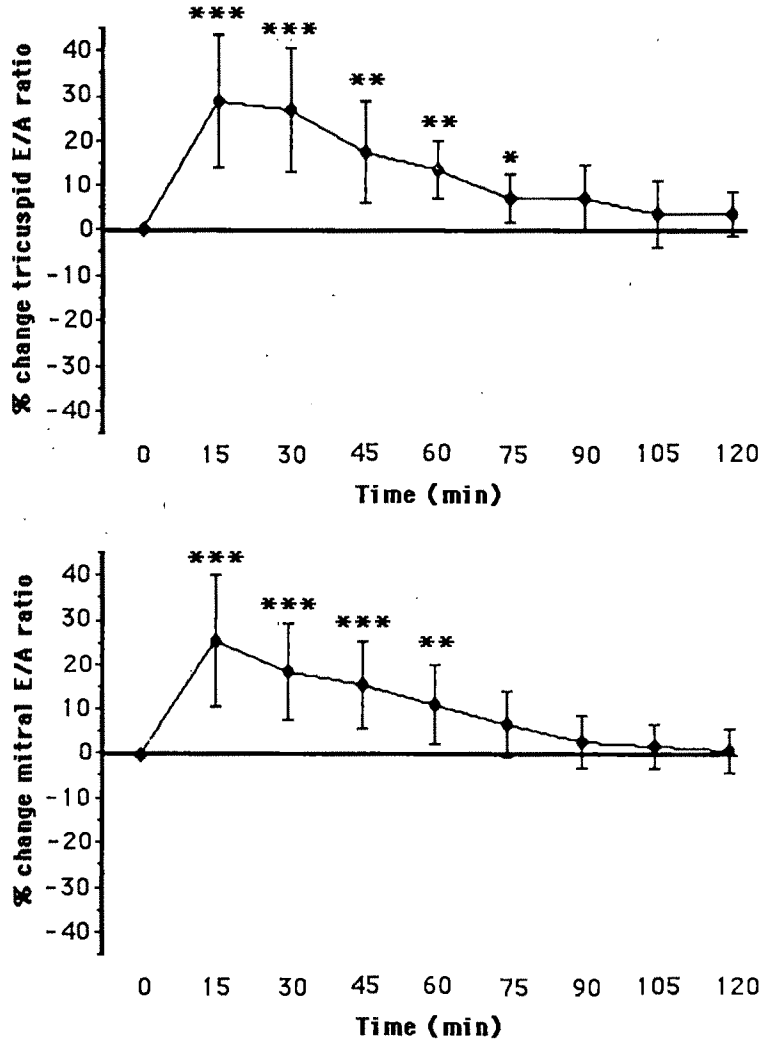


Fig. 4. Mean percentage changes (± 1 SD) of transtricuspid and transmitral E/A ratios after transfusion. Differences on comparison with pretransfusion values were evaluated by means of the paired Student *t* test (* = $p \leq 0.01$; ** = $p \leq 0.002$; *** = $p \leq 0.001$).

Posttransfusion (+30 min)						*Posttransfusion (+120 min)*					
Tricuspid valve			*Mitral valve*			*Tricuspid*			*Mitral valve*		
RCO	*HR*	*E/A*	*LCO*	*HR*	*E/A*	*RCO*	*HR*	*E/A*	*LCO*	*HR*	*E/A*
381	149	1.02	290	147	1.02	444	143	0.97	367	145	0.94
553	135	1.26	459	128	1.20	803	135	0.94	—	—	—
595	130	1.17	438	133	1.08	737	125	0.97	523	122	0.92
645	150	1.18	467	150	1.04	877	146	0.89	636	150	0.90
265	150	1.19	284	150	1.10	—	—	—	—	—	—
459	133	0.97	369	125	1.08	682	135	0.92	722	140	1.02
338	158	1.15	288	162	1.17	416	140	0.92	348	130	0.94
769	120	1.19	744	133	1.09	969	128	1.00	1017	120	0.95
430	153	1.04	224	133	0.98	467	130	0.95	387	135	0.87
381	147	1.09	308	142	1.07	581	141	0.80	507	144	0.87
594	139	0.97	601	138	0.85	936	131	0.87	847	129	0.84
504	138	1.12	351	133	1.07	728	133	0.96	572	140	0.87

ilar to that described in hypoxic growth-retarded fetuses (i.e., the so-called "brain sparing effect").[7] These findings suggest that in red blood cell isoimmunization the changes in fetal cardiac output are mainly related to the low blood viscosity.

Intravascular fetal blood transfusion was associated with a rapid decrease in cardiac output that was primarily the result of decrease in stroke volume rather than heart rate. These changes were similar in both ventricles and reached a nadir at 15 to 45 minutes after the transfusion. Thereafter the cardiac output increased but still remained below the pretransfusion level.

These findings are compatible with the observations of Bilardo et al.[4] who measured the time-averaged mean velocity in the fetal descending thoracic aorta and common carotid artery before and within 30 minutes of intravascular transfusion and found a significant decrease in both vessels.

A possible explanation for posttransfusion decrease in cardiac output is reduced venous return to the heart secondary to the increased hematocrit and therefore viscosity of blood or secondary to the release of vasodilators such as prostacyclin or atrial natriuretic factor.[16, 17] However, this is unlikely because the relative E/A ratios found after the transfusion suggest that cardiac preload is increased rather than decreased. Furthermore, if the decrease in cardiac output was because of increased hematocrit, this effect would be sustained for at least 24 hours. Copel et al.[12] measured velocities in the aorta and also cardiac output at 12 hours after intravascular blood transfusion and found no significant differences from the pretransfusion levels. Finally, the reported increase of umbilical venous pressure after intravascular transfusion[18, 19] does not support the hypothesis of a venous vasodilatation as the cause of a reduction in venous return.

The alternative explanation for the posttransfusion fall in cardiac output is cardiovascular overload. This is supported by the findings of a direct relationship between the fall in cardiac output and the degree of expansion of the fetoplacental blood volume. Animal studies have shown that the fetal heart has very limited reserve capacity to increase its output in response to acute overload[18] and that massive increases in fetal blood volume are associated with a decrease in cardiac output.[19] After a transfusion there is a rapid rate of fluid loss[20] and this explains the rapid recovery in E/A ratios and cardiac output.

Doppler echocardiography provides a reproducible method for the measurement of cardiac output in the human fetus.[5, 6] However, this analysis is prone to errors mainly because of inaccuracies in the determination of valve diameters that are halved and squared in the calculation of cardiac output and thus amplified. Nevertheless in our investigation the flow orifice area is assumed to remain unmodified and therefore potential errors in assessment of valve diameters would affect similarly the measurements performed during the study without impairing the reliability of the results obtained.

In conclusion, fetal anemia is associated with increased cardiac output. Rapid intravascular blood transfusion causes a temporary and symmetrical decrease in right cardiac output and left cardiac output that is proportional to the degree of expansion in fetoplacental blood volume.

REFERENCES

1. Nicolaides KH, Soothill PW, Rodeck CH. Rh disease: intravascular fetal blood transfusion by cordocentesis. Fetal Ther 1986;1:185-92.
2. Nicolaides KH, Clewell WH, Rodeck CH. Measurement of human fetoplacental blood volume in erythroblastosis fetalis. AM J OBSTET GYNECOL 1987;157:50-3.
3. Copel JA, Grannum PA, Belanger K, Green J, Hobbins

JC. Pulsed Doppler flow velocity waveforms before and after intrauterine intravascular transfusion for severe erythroblastosis fetalis. Am J Obstet Gynecol 1988; 158:768-74.
4. Bilardo CM, Nicolaides KH, Campbell S. Doppler studies in red cell isoimmunization. Clin Obstet Gynecol 1989; 32:719-27.
5. Reed KL, Meijboom EJ, Sahn DJ, Scagnelli SA, Valdes-Cruz LM, Skenker L. Cardiac Doppler flow velocities in human fetuses. Circulations 1986;73:41-56.
6. De Smedt MCH, Visser GHA, Meijboom EJ. Fetal cardiac output estimated by Doppler echocardiography during mid- and late gestation. Am J Cardiol 1987;338-42.
7. Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal haemoglobin measurement in the assessment of red cell isoimmunization. Lancet 1988;1:1073-5.
8. Rizzo G, Arduini D, Romanini C, Mancuso S. Doppler echocardiographic assessment of atrioventricular velocity waveforms in normal and small for gestational age fetuses. Br J Obstet Gynaecol 1988;95:65-9.
9. Shepard MJ, Richards VA, Berkowitz RL, Warsof SL, Hobbins JC. An evaluation of two equations for predicting fetal weight by ultrasound. Am J Obstet Gynecol 1982;142:47-54.
10. Soothil PW, Nicolaides KH, Rodeck CH, Campbell S. Effects of gestational age of fetal and intervillous blood gas and acid-base values in the human pregnancy. Fetal Ther 1986;1:168-75.
11. Fumia FD, Edelstone DI, Holzman IR. Blood flow and oxygen delivery as functions of fetal hematocrit. Am J Obstet Gynecol 1984;150:274-82.
12. Copel JA, Grannum PA, Green JJ, et al. Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion. Am J Obstet Gynecol 1989;161:361-4.
13. Visser GHA, De Smedt MCH, Meijboom EJ. Altered fetal cardiac flow patterns in pure red cell anemia (the Blackfan-Diamond syndrome). Prenat Diagn 1988;8: 525-9.
14. Kirkinen P, Jouppila P. Eik-Nes S. Umbilical vein blood flow in rhesus isoimmunization. Br J Obstet Gynaecol 1983;90:640-3.
15. Rightmire DA, Nicolaides KH, Rodeck CH, Campbell S. Fetal blood velocities in Rh isoimmunization: relationship to gestational age and to fetal hematocrit. Obstet Gynecol 1986;68:233-6.
16. Weiner CP, Robillard JE. Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations. Am J Obstet Gynecol 1989;161:1494-7.
17. Panos MZ, Nicolaides KH, Anderson JV, Economides DL, Rees L, Williams R. Plasma atrial natriuretic peptide in the human fetus: response to intravascular blood transfusion. Am J Obstet Gynecol 1989;161:357-60.
18. Nicolini U, Talbert DG, Fisk NM, Rodeck CH. Pathophysiology of pressure changes during intrauterine transfusion. Am J Obstet Gynecol 1989;160:1139-45.
19. Weiner CP, Pelzer GD, Heilskov J, Wenstrom KD, Williamsons RA. The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops. Am J Obstet Gynecol 1989;161:1489-501.
20. Gilbert RD. Control of fetal cardiac output during changes in blood volume. Am J Physio 1980;238:H80-6.
21. Heymann MA, Rudolph AM. Effects of increasing preload on right ventricular output in fetal lambs in utero. Circulation 1973;48(suppl 4):37.
22. Brace RA. Fetal blood volume response to intravenous saline solution and dextrane. Am J Obstet Gynecol 1983;143:777-81.

Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy

Jim van Eyck, MD, PhD, Patricia A. Stewart, PhD, and Juriy W. Wladimiroff, MD, PhD
Rotterdam, The Netherlands

In 16 normal pregnancies the relationship between foramen ovale flow velocity waveforms and fetal behavioral states at 37 to 38 weeks' gestation was studies with Doppler techniques. Foramen ovale flow velocity waveforms are characterized by a typical systolic/diastolic component. Average flow velocity was statistically significantly increased in behavioral state 2F (active sleep) as compared with state 1F (quiet sleep), which was mainly determined by an increase in flow velocity during the end-systolic and passive atrial filling phase. The increase in the average flow velocity at the foramen ovale level during active sleep suggests a redistribution of blood flow at the cardiac level, resulting in an increased right-to-left shunt. (Am J Obstet Gynecol 1990;163:1239-42.)

Key words: Human fetal behavioral states, foramen ovale flow velocity, fetal Doppler ultrasonography

In the normal growing human fetus at term blood flow velocity waveforms obtained from the lower thoracic part of the fetal descending aorta[1] and internal carotid artery[2] show fetal behavioral state dependency. In state 2F (active sleep) pulsatility index values in these vessels are significantly reduced as compared with state 1F (quiet sleep), reflecting reduced downstream impedence and suggesting increased perfusion of skeletal musculature and cerebrum during active sleep.[1, 2] At the cardiac level it was demonstrated that in the fetal ductus arteriosus peak flow velocity during behavioral state 2F is markedly reduced as compared with state 1F, suggesting a redistribution in left and right ventricular output in favor of the left side of the heart.[3]

In the normal fetus, inferior vena cava flow represents 65% to 70% of the total venous return to the heart,[4] approximately 40% of which is shunted to the left atrium through the foramen ovale. Behavioral state–related changes in foramen ovale flow could account for the suggested redistribution of ventricular output. With the introduction of combined realtime and Doppler systems the possibility became available to study blood flow velocity waveforms at the level of the foramen ovale. The objective of the present study was to investigate the relationship between blood flow velocity waveforms at the level of the foramen ovale and behavioral state 1F and 2F according to the classification of Nijhuis et al.[5] in the normal term fetus.

From the Department of Obstetrics and Gynecology, Academic Hospital Rotterdam-Dijkzigt.
Received for publication January 30, 1990; revised May 24, 1990; accepted June 15, 1990.
Reprint requests: Juriy W. Wladimiroff, MD, PhD, Department of Obstetrics and Gynecology, Academic Hospital Rotterdam-Dijkzigt, Erasmus University Rotterdam, Dr. Molewaterplein 40, Rotterdam, The Netherlands 3015 GD.
6/1/23229

Material and methods

A total of 16 women with normal singleton pregnancies at 37 to 38 weeks' gestation gave consent to participate in the study. The gestational age had been calculated from a reliable menstrual history and early ultrasonographic measurement of fetal crown-rump length or biparietal diameter. Fetal birth weight was between the 10th and 90th percentile for gestational age, according to Kloostermans' tables, corrected for maternal parity and fetal sex.[6] All subjects were nonsmokers; no medications were prescribed. All studies were carried out 2 hours following after breakfast or lunch, with the study participants in the semirecumbent position. With a two-dimensional real-time mechanical sector scanner (Vingmed CFM 700, Sonotron, Horten, Norway; carrier frequency 3 MHz, Doppler 3 MHz with variable depth of sample gate and sample size) blood flow velocity recordings through the foramen ovale were made with either a four-chamber view or a modified short-axis view. In both views the valve of the foramen ovale could always be visualized. Color Doppler techniques were used in the first two participants but were subsequently abandoned as the central portion of the foramen ovale "defect" was clearly visualized with standard cross-sectional techniques. The sample gate was placed across the septal opening and the angle of insonation was always kept below 10°. Flow velocity waveforms at the foramen ovale level were obtained during behavioral states 1F and 2F, which were defined as follows:

State 1F: quiescence, which can be regularly interrupted by brief gross body movements, which mostly

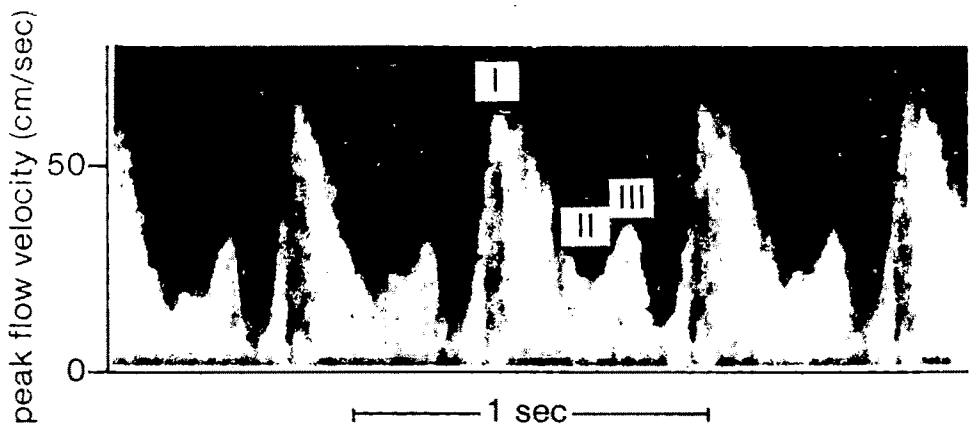


Fig. 1. Foramen ovale flow velocity waveforms *I* = systolic ventricular phase, *II* = end-systolic ventricular phase, and *III* = passive atrial filling phase.

are startles; eye movements absent and a stable heart rate pattern with a small oscillation bandwidth. Isolated accelerations do occur, but are strictly related to fetal movements.

State 2F: frequent and periodic gross body movements that are mainly stretches and retroflexions and movements of extremities; eye movements continually present and a heart rate pattern with a wider oscillation bandwidth than in state 1F and frequent accelerations during movements.

To establish these behavioral states the following parameters were simultaneously recorded: (1) fetal heart rate, which was obtained from a Doppler ultrasonographic cardiotocograph (Hewlett Packard 8040A, Boeblingen, Germany; carrier frequency 1 MHz); (2) fetal eye movements, which were studied from a transverse view of the fetal face with a two-dimensional real-time linear array scanner (Hitachi EUB-27, carrier frequency 3.5 MHz; and (3) fetal body movements, which were obtained from a sagittal view of the fetal trunk with a two-dimensional real-time and mechanical sector scanner (Vingmed CFM 700, carrier frequency 3 MHz).

The three transducers were placed in such a way that there was minimal interference between the three ultrasonographic modes. Flow velocity recordings were only performed when a clear fetal behavioral state was identified and when this state had been present over a period of at least 3 minutes. All recordings were performed during fetal apnea. The maximum amount of time for the completion of a flow velocity recording after a state determination was 3 minutes. The blood flow velocity waveforms were recorded on videotape. From hard copies of each recording, optimal flow velocity waveforms were selected. The foramen ovale waveform consists of two components (Fig. 1). During ventricular systole flow velocity reaches a maximum (I), after which it reduces in late systole (II). During the passive atrial filling phase corresponding with the E wave again there is an increase in flow velocity (III) that decreases with atrial contraction to zero (D. Soyeur et al. 1989, personal communication). With a microcomputer (Olivetti M24) flow velocities (centimeters per second) were calculated during the peak systolic (I), end-systolic (II), and passive atrial filling phase (III) of the cardiac cycle. Moreover, the average velocity (centimeters per second) and period time (cycle duration in milliseconds) were determined. To reject the null hypothesis that there is no difference in the individual paired differences of waveform components between behavioral state 1F and 2F, we used the two-tailed Student *t* test. Statistical significance was considered to be present if $p < 0.05$.

Results

Doppler signals could not obtained in two women because of anterior position of the fetal spine, leaving 14 women for further analysis. The mean number of blood flow velocity waveforms studied at the level of the fetal foramen ovale for all 14 women was 17 ± 8 (1 SD) in behavioral state 1F and 16 ± 5 (1 SD) in behavioral state 2F, resulting in a total number of 460 waveforms. Measurements of peak systolic, end-systolic, passive atrial filling phase, and average flow velocity showed considerable consistency reflected by the mean value of the SD of the individual measurements being 2.4, 1.6, 2.6, and 1.9 for behavioral state 1F and 3.1, 2.1, 2.4, and 1.8 for behavioral state 2F. The results of the paired analysis of the data in behavioral states 1F and 2F are presented in Table I. Average flow velocity was significantly ($p < 0.001$) increased in behavioral state 2F as compared with state 1F. This was mainly determined by an increase in flow velocity during the end-systolic and passive atrial filling phase of the foramen ovale flow velocity waveform (Fig. 2). No significant difference in period time could be established between behavioral states 1F and 2F.

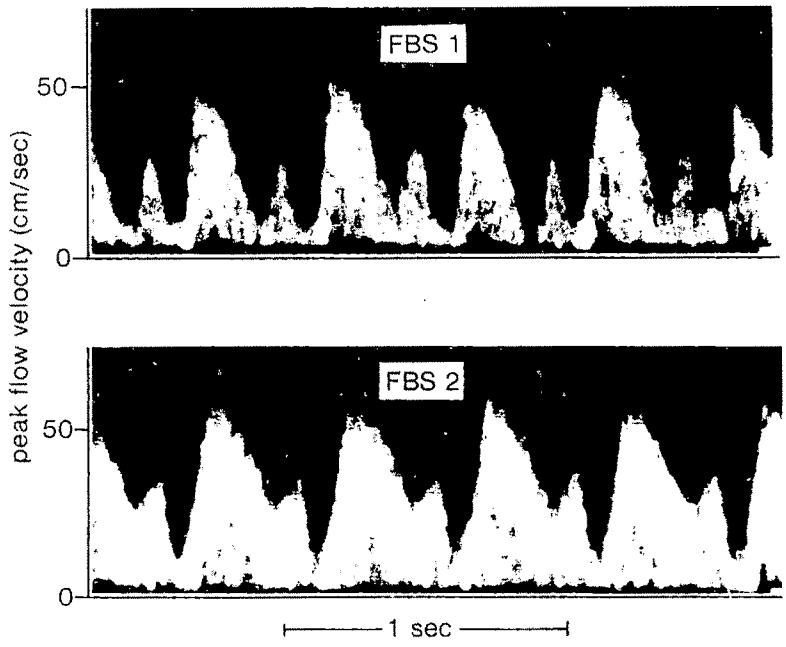


Fig. 2. Foramen ovale flow velocity waveforms obtained during behavioral state 1F *(upper panel)* and state 2F *(lower panel)*.

Table I. Foramen ovale flow velocity waveform parameters relative to behavioral state 1F and 2F

	No. of paired observations	*Behavioral state*		*Difference**	*SD†*	p *Value*
		1F (mean value)	*2F (mean value)*			
Period time (msec)	14	430.6	428.9	−1.7	16.6	NS
Average velocity (cm/sec)	14	22.6	27.6	5.0	2.6	<0.001
PV I (cm/sec)	14	42.8	46.8	4.0	7.4	NS
PV II (cm/sec)	14	10.0	18.7	8.7	2.6	<0.001
PV III (cm/sec)	14	23.7	28.2	4.5	3.8	<0.001

PV I, Peak velocity during systolic ventricular phase; *PV II*, peak velocity during end-systolic ventricular phase; *PV III*, peak velocity during passive atrial filling phase.

*Mean paired difference between behavioral state 2F and behavioral state 1F.

†SD of the mean paired difference.

Comment

Doppler ultrasonography allows visualization of blood flow through the foramen ovale and documentation of flow velocity waveforms through this cardiac shunt in the normal term fetus. During fetal development there are two major shunts at the cardiac level (i.e., the ductus arteriosus and the foramen ovale). In the fetal ductus arteriosus it was demonstrated that peak systole flow velocity is markedly reduced during behavioral state 2F as compared with state 1F, suggesting a redistribution in left and right ventricular output in favor of the left side of the heart.[3]

The results of the present study show that peak flow velocity through the foramen ovale is nearly twice as high during ventricular systole as compared with the diastolic phase. The increased foramen ovale flow during ventricular contraction may be explained by the fact that the closed tricuspid valve prevents superior vena cava blood flow from entering the right ventricle, leading to a raised right-to-left atrial pressure gradient. The upward movement of the tricuspid leaflets during ventricular contraction further contributes to this increased pressure gradient. Moreover, averaged flow velocities at the foramen ovale level are raised in behavioral state 2F. Because volume flow is equal to the average flow velocity multiplied by area, the increase in the average flow velocity could be a result of an increase in volume flow through the foramen ovale during active sleep. Inasmuch as the foramen ovale will open at an increased pressure gradient between the right and left atrium, one may speculate that flow velocity will be negatively influenced by the subsequent

increase in lumen area of the formen ovale. Therefore volume flow through the foramen ovale may be even larger than the rise in flow velocity would suggest. The described flow changes at the foramen ovale level are in agreement with the behavioral state–dependent flow changes documented in the ductus arteriosus[3] in that they suggest that in the normal term fetus the active sleep state is associated with preferential flow to the left side of the heart. Elevated average flow velocities at the foramen ovale level are mainly determined by the end-systolic and passive atrial filling phase of the flow velocity waveform, suggesting increased passive atrial filling during diastole as a result of a raised preload. This would be in agreement with Teitel et al.[7] who showed that during fetal development venous return (preload) can be raised by an increased preferential streaming of umbilical venous blood through the ductus venosus into the inferior vena cava, by-passing the hepatic microcirculation.

It can be concluded that in the normal term fetus the foramen ovale plays an important role in behavioral state–dependent redistribution of blood flow at the cardiac level.

REFERENCES

1. van Eyck J, Wladimiroff JW, Noordam MJ, Tonge HM, Prechtl HFR. The blood flow velocity waveform in the fetal descending aorta; its relationship to fetal behavioral states in normal pregnancy at 37-38 weeks. Early Hum Dev 1985;12:137-43.
2. van Eyck J, Wladimiroff JW, van den Wijngaard JAGW, Noordam MJ, Prechtl HFR. The blood flow velocity waveform in the fetal internal carotid and umbilical artery; its relationship to fetal behavioral states in normal pregnancy at 37-38 weeks of gestation. Br J Obstet Gynaecol 1987; 94:736-41.
3. van der Mooren K, van Eyck J, Wladimiroff JW. Human fetal ductal flow velocity waveforms relative to behavioral states in normal term pregnancy. AM J OBSTET GYNECOL 1989;160:371-4.
4. Rudolph AM. Changes in the circulation after birth. In: Congenital diseases of the heart. Chicago: Year Book, 1974:17-28.
5. Nijhuis JG, Prechtl HFR, Martin Jr CB, Bots RSGM. Are there fetal behavioural states in the human fetus? Early Hum Dev 1982;6:177-95.
6. Kloosterman G. On intrauterine growth. Int Gynaecol Obstet 1970;8:895-912.
7. Teitel D, Rudolph AM. Perinatal oxygen delivery and cardiac function. Adv Pediatr 1985;32:321-47.

Suppression of serum insulin level by diazoxide does not alter serum testosterone or sex hormone–binding globulin levels in healthy, nonobese women

John E. Nestler, MD,[a] Roshnara Singh, MD,[a] Dennis W. Matt, PhD,[b] John N. Clore, MD,[a] and William G. Blackard, MD[a]

Richmond, Virginia

Suppression of serum insulin levels with diazoxide is associated with a decrease in serum testosterone and an increase in serum sex hormone–binding globulin in obese women with the polycystic ovary syndrome. To determine whether physiologic insulin levels play a regulatory role in the androgen status of nonobese women with normal menses, the androgen status of five nonobese normal women was assessed on two occasions: during a control study and after 10 days of oral diazoxide (100 mg, three times daily) administration. Insulin release in response to 100 gm oral glucose administration decreased from 108.0 ± 28.2 to 49.3 ± 5.2 nmol · min/L ($p = 0.05$) after diazoxide administration. However, despite suppression of insulin release, diazoxide administration did not affect serum total testosterone (diazoxide, 0.73 ± 0.10; control, 0.69 ± 0.11 nmol/L; p = NS) or sex hormone–binding globulin (diazoxide, 79.7 ± 16.6; control, 70.2 ± 12.6 nmol/L; p = NS) concentrations. These observations suggest that physiologic insulin levels in nonobese healthy women do not regulate testosterone metabolism and that diazoxide does not exert a direct or independent effect on serum testosterone or sex hormone–binding globulin levels. (Am J Obstet Gynecol 1990;163:1243-6.)

Key words: Polycystic ovary syndrome, testosterone, insulin resistance, androgens, hyperinsulinemia

The hyperandrogenism of some women with the polycystic ovary syndrome may result from hyperinsulinemia.[1] In support of this view we recently demonstrated that suppression of serum insulin levels with diazoxide is associated with a fall in serum testosterone levels and a rise in serum sex hormone–binding globulin levels in obese women with the polycystic ovary syndrome.[2] However, it remains unknown whether physiologic insulin levels play a regulatory role in the androgen status of nonobese women with normal menses or whether diazoxide itself might directly affect androgen homeostasis. To address these issues, we assessed the androgen status of five nonobese healthy women during a control period and after suppression of serum insulin levels with diazoxide.

From the Departments of Internal Medicine[a] and Obstetrics and Gynecology,[b] Medical College of Virginia/Virginia Commonwealth University.
Supported in part by National Institutes of Health grants RR-00065 and DK-18904, a Research Award from the Virginia Affiliate of the American Diabetes Association, and the Thomas F. and Kate Miller Jeffress Memorial Trust.
Received for publication February 20, 1990; revised May 23, 1990; accepted June 1, 1990.
Reprint requests: John E. Nestler, MD, Division of Endocrinology and Metabolism, Medical College of Virginia, MCV Station, Box 111, Richmond, VA 23298-0111.
6/1/22840

Material and methods

Study design. Eight women were initially enrolled into the study but three women withdrew before completion because of side effects of diazoxide (diffuse edema, palpitations, anxiety). The remaining five women were between 23 and 39 years old and nonobese (body mass index values ranged from 18.5 to 24.5 kg/m^2). Each woman was healthy, had a normal menstrual history, and was not taking any medications. The study was approved by the Committee on the Conduct of Human Research of the Medical College of Virginia, and signed informed consent was obtained from each subject.

All subjects were studied in the follicular phase of the menstrual period (between days 5 and 9 after onset of menses). Two subjects were studied during a control period and once again 28 days later after having taken 100 mg diazoxide orally three times daily (300 mg/day) for 10 days. The remaining three subjects were studied in the reverse order. The subjects were allowed to eat at will, and the last dose of diazoxide was taken on the morning of study.

The control study and the study after diazoxide administration were conducted in an identical manner. The women were admitted to the General Clinical Research Center of the Medical College of Virginia after

an overnight fast. Weight and height were recorded, and a heparin lock was inserted into a hand vein for blood withdrawal. Blood samples were drawn at 8, 8:15, and 8:30 AM, and the sera were pooled for determination of serum steroid, sex hormone–binding globulin, and gonadotropin concentrations. To determine the efficacy of diazoxide in inhibiting insulin release, the women drank 100 gm of glucose (Glucola, Whitaker General Medical, Richmond, Va.) at 8:30 AM, and blood samples were drawn every 15 minutes from 8:30 to 11:30 AM for determination of serum glucose and insulin levels.

Assays. Serum glucose concentrations were determined by the glucose oxidase method (Beckman Glucose Analyzer 2, Fullerton, Calif.). Serum insulin levels were determined by radioimmunoassay.[3] The intraassay coefficient of variation for the insulin assay was 5.0%. The integrated insulin response during the oral glucose tolerance test was analyzed by calculating the area under the curve for insulin during the 3 hours after glucose ingestion by the trapezoidal rule using absolute values.

Serum testosterone, androstenedione, estrone, 17β-estradiol, and progesterone concentrations were measured by radioimmunoassay as previously described.[2, 4] Serum (1.0 ml) was extracted with diethyl ether, and steroids were isolated by Celite column chromatography. Highly specific antisera were used to measure testosterone, androstenedione, estrone, 17β-estradiol, and progesterone. Tritium-labeled steroids were added to serum before extraction to correct for procedural losses. The antiserum to testosterone was provided by Dr. Gordon Niswender (Department of Physiology, Colorado State University, Fort Collins, Colo.), and the antisera to androstenedione, 17β-estradiol, and progesterone were provided by Dr. John Resko (Department of Physiology, University of Oregon School of Medicine, Portland, Ore.). Antiserum to estrone was purchased from Steranti Research Ltd. (London). The intraassay coefficients of variation for all the steroid assays were <8.0%. Serum sex hormone–binding globulin concentrations were determined by an immunoradiometric assay with a commercial kit (Diagnostic Products, Los Angeles), and the intraassay coefficient of variation was 2.5%. Serum luteinizing hormone and follicle-stimulating hormone concentrations were determined by radioimmunoassay (Pacific Biotech, San Diego). The intraassay coefficients of variation of luteinizing hormone were 10.4% for 5 to 8 IU/L, 12.1% for 8 to 11 IU/L, and 6.6% for 11 to 35 IU/L. The intraassay coefficient of variation of follicle-stimulating hormone was 6.5%.

Glucose, gonadotropin, and sex hormone–binding globulin measurements from each woman were determined in duplicate in single assays.

Statistical analysis. All results are reported as the mean ± SE. All results were first tested for normality with the Wilk-Shapiro test and were then analyzed by Student's two-tailed paired t test. A value of $p < 0.05$ was considered significant.

Results

Mean body mass index increased by 0.8 kg/m² in the five women during diazoxide administration (diazoxide, 22.4 ± 1.3 kg/m²; control, 21.6 ± 1.3 kg/m²; $p < 0.015$), most likely as a result of fluid retention. In four women pretibial edema developed while they were taking diazoxide. Body mass index values returned to baseline shortly after cessation of diazoxide administration.

During the control study the women demonstrated normal glycemic and insulin responses during the oral glucose tolerance test (Fig. 1). Diazoxide administration uniformly decreased insulin release (area under the curve for insulin: diazoxide, 49.3 ± 5.2 nmol · min/L; control, 108.0 ± 28.2 nmol · min/L; $p = 0.05$) and caused deterioration of glucose tolerance (Fig. 1). Diazoxide administration did not significantly alter mean fasting serum insulin (diazoxide, 76 ± 25 pmol/L; control, 115 ± 19 pmol/L; p = NS) or glucose (diazoxide, 5.4 ± 0.4 mmol/L; control, 4.5 ± 0.1 mmol/L; p = NS) concentrations.

Diazoxide administration did not affect serum total testosterone or sex hormone–binding globulin concentrations (Table I). Diazoxide administration also did not alter serum androstenedione, estrone, 17β-estradiol, progesterone, luteinizing hormone, or follicle-stimulating hormone levels (Table I).

Comment

Hyperinsulinemic insulin resistance may be the cause of hyperandrogenism in some women with the polycystic ovary syndrome.[1] In support of this hypothesis, insulin resistance and elevated serum insulin levels have been well documented in women with the polycystic ovary syndrome.[5-7] This insulin resistance does not appear to be solely a result of the obesity often associated with the polycystic ovary syndrome because nonobese women with the polycystic ovary syndrome also manifest hyperinsulinemic insulin resistance.[7, 8]

To examine the role of chronic hyperinsulinemia on androgen levels in this disorder, we previously used diazoxide to reduce serum insulin levels in obese women with the polycystic ovary syndrome.[2] Diazoxide treatment uniformly suppressed glucose-stimulated insulin release and resulted in a significant reduction in serum testosterone concentrations. Serum sex hormone–binding globulin levels rose in all subjects. Since gonadotropin release did not change as a result of diazoxide administration, these results support the

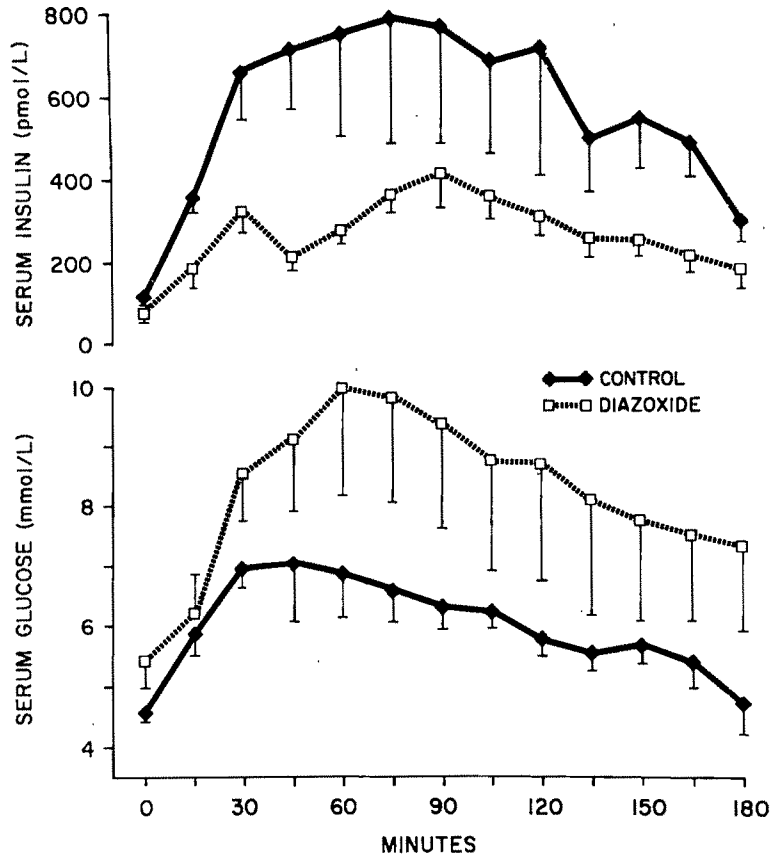


Fig. 1. Mean (± SE) serum glucose and insulin responses after 100 gm of oral glucose in five nonobese normal women during the control study *(closed circles)* and after 10 days of oral diazoxide (300 mg/day) administration *(open boxes)*.

Table I. Serum steroid, gonadotropin, and sex hormone–binding globulin concentrations in five nonobese, normal women at the time of the control study and after 10 days of oral diazoxide (300 mg/day) administration

	Control	*Diazoxide*	*p Value*
Testosterone (nmol/L)	0.69 ± 0.11	0.73 ± 0.10	0.71
Androstenedione (nmol/L)	3.5 ± 0.4	3.5 ± 0.3	0.96
Estrone (pmol/L)	296 ± 29	456 ± 56	0.09
17β-Estradiol (pmol/L)	270 ± 49	288 ± 42	0.40
Progesterone (nmol/L)	0.16 ± 0.03	0.09 ± 0.03	0.20
Luteinizing hormone (IU/L)	12.0 ± 1.1	11.2 ± 1.5	0.66
Follicle-stimulating hormone (IU/L)	9.7 ± 0.4	10.3 ± 0.9	0.62
Sex hormone–binding globulin (nmol/L)	70.2 ± 12.6	79.7 ± 16.6	0.24

Values represent mean ± SE.

hypothesis that hyperinsulinemia can directly affect testosterone metabolism.

In marked contrast, neither serum testosterone nor sex hormone–binding globulin levels changed in this study when serum insulin levels of nonobese healthy women were suppressed with diazoxide. These observations suggest either that normal women are not as sensitive to actions of insulin on androgen homeostasis as are women with the polycystic ovary syndrome or that insulin concentrations in the physiologic range do not play an important role in regulation of serum testosterone levels. Although the number of subjects in this study equaled the number of obese women with the polycystic ovary syndrome previously examined,[2] it remains possible that the negative results of this study were a result of the limited number of subjects.

In support of the possibility that normal women are not as sensitive to the androgenic actions of insulin as

are women with the polycystic ovary syndrome, evidence exists suggesting that androgenic pathways of normal women may be less responsive to stimulation by insulin than those of women with the polycystic ovary syndrome. Ovarian tissues removed from women with hyperandrogenism exhibit enhanced androstenedione and testosterone release in response to insulin, whereas ovarian tissues removed from normal women remain unaffected by insulin treatment.[9] This disparity also was noted in an in vivo study that demonstrated various effects of suprapharmacologic hyperinsulinemia on sex steroid concentrations of women with the polycystic ovary syndrome but not in normal women.[10]

Alternatively, it has been postulated that in the polycystic ovary syndrome elevated serum insulin levels affect androgenic pathways by cross reacting with the insulin-like growth factor-I receptor.[11] Insulin concentrations in the physiologic range may be too low to significantly associate with insulin-like growth factor-I receptors. Thus insulin may not regulate serum androgen levels under physiologic conditions.

Finally, these observations also indicate that diazoxide does not directly affect serum testosterone or sex hormone–binding globulin levels in vivo. Thus the androgen changes observed when diazoxide is administered to obese women with the polycystic ovary syndrome[2] are indeed a result of suppression of insulin release and are not an independent effect of diazoxide.

In summary, suppression of serum insulin levels by diazoxide in nonobese healthy women does not affect serum testosterone or sex hormone–binding globulin levels. These observations suggest that physiologic insulin levels in normal women do not regulate androgenic processes.

We thank the staff of the General Clinical Research Center of the Medical College of Virginia; Ms. Bettie Duke, CLINFO systems analyst; and Dr. Vernon Chincilli of the Department of Biostatistics for their help.

REFERENCES

1. Nestler JE, Clore JN, Blackard WG. The central role of obesity (hyperinsulinemia) in the pathogenesis of the polycystic ovary syndrome. Am J Obstet Gynecol 1989; 161:1095-7.
2. Nestler JE, Barlascini CO, Matt DW, et al. Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 1989;68:1027-32.
3. Morgan CR, Lazarow A. Immunoassay of insulin: two antibody system. Diabetes 1963;12:115-26.
4. DeVane GW, Czekala NM, Judd HL, Yen SSC. Circulating gonadotropins, estrogens and androgens in polycystic ovarian disease. Am J Obstet Gynecol 1975;121:496-500.
5. Burghen GA, Givens JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50:113-6.
6. Shoupe D, Kumar DD, Lobo RA. Insulin resistance in polycystic ovary syndrome. Am J Obstet Gynecol 1983; 147:588-92.
7. Chang RJ, Nakaruma RM, Judd HL, Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metab 1983;57:356-9.
8. Dunaif A, Mandeli J, Fluhr H, Dobrjansky A. The impact of obesity and chronic hyperinsulinemia on gonadotropin release and gonadal steroid secretion in the polycystic ovary syndrome. J Clin Endocrinol Metab 1988;66: 131-9.
9. Barbieri RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab 1986; 62:904-10.
10. Dunaif A, Graf M. Insulin administration alters gonadal steroid metabolism independent of changes in gonadotropin secretion in insulin resistant women with the polycystic ovary syndrome. J Clin Invest 1989;83:23-9.
11. Poretsky L, Kalin MF. The gonadotropic function of insulin. Endocr Rev 1987;8:132-41.

Intrapartum uterine rupture and inflammatory bowel disease

Ila M. Peterson, MD, and Bev Braak, MD
Kansas City, Missouri

The incidence of uterine rupture in pregnant women ranges from 0% to 1.1% in large series. This case report describes a woman with a history of obstetric complications, uterine rupture, and active inflammatory bowel disease that was treated with high-dose prednisone. (AM J OBSTET GYNECOL 1990;163:1247-8.)

Key words: Uterine rupture, inflammatory bowel disease, prednisone

Case report

A 24-year-old white woman (gravida 4, para 1-0-1-1) had a 10-year history of active Crohn's disease treated with 40 to 60 mg of prednisone daily. She was seen at 33 weeks' gestation (by dates and ultrasonography) complaining of nausea, vomiting, diarrhea, and contractions for several days. This pregnancy had been complicated by several admissions for exacerbation of regional enteritis and preterm labor. A previous pregnancy 13 months earlier resulted in a classic cesarean section at 27 weeks' gestation for abruptio placentae; the infant did not survive. In 1984 the patient was vaginally delivered of a premature infant at 34 weeks' gestation; that child is alive and well. Past surgical history was significant for colonic and partial small bowel resection in 1984 for Crohn's enteritis. The patient also had documented iron-deficiency anemia and marasmus malnutrition on multiple previous hospital admissions.

On admission the patient was pale and listless but her vital signs were normal. She had a right-sided draining perianal fistula and multiple perianal scars. The cervix was dilated to 1 cm and thick. Fetal heart tones were reactive and without decelerations. Contractions every 2 to 4 minutes were present on monitor; they failed to resolve after administration of intravenous ritodrine and only gradually decreased in frequency with use of magnesium sulfate. A bedside scan showed fetal breech presentation and an anterior placenta. By the fourth hospital day, the patient was given oral ritodrine without incident; the perianal fistula had improved after treatment with metronidazole (Flagyl, Searle & Co., Chicago). On the seventh hospital day the patient had mild regular contractions unchanged by hydration and sedation. She suddenly experienced severe, relentless abdominal pain; the abdomen grew tense and tender, and there was a small amount of blood present in the vagina. Fetal heart tones dropped from the range of 140 beats/min to the range of 80 beats/min and were unresponsive to oxygen and position change. An immediate cesarean section was performed for suspected abruptio placentae. The fetus was in its intact amniotic sac in the abdominal cavity. The anterior wall of the uterus was ruptured vertically in the midline and dark clots were seen at the margins of the myometrial wound. A 1788 gm male infant with Apgar scores of 0 at 1 minute and 3 at 5 minutes and a venous cord pH of 6.84 was delivered. Subsequently a total abdominal hysterectomy was performed. Mother and child were discharged in good condition on postoperative day 18.

The uterus with attached cervix weighed 435 gm. A large anterior vertical 10 cm uterine rupture was present and its margins were thick and hemorrhagic. The dehiscence was 6 cm wide. The uterine fundal wall was 3 cm thick and the region of the lower uterine segment was markedly thinned to 5 mm. Multiple sections through the area of the ruptured anterior wall showed focal hyalinization of smooth muscle, no necrosis, and no granulation tissue. Dense scarification was absent. Sections of the 1.2 cm, patent, intact cervix showed widespread human papillomavirus effect and moderate dysplasia. The discoid placenta was complete and weighed 407 gm, its trivesseled umbilical cord was eccentrically inserted and the placental membranes were cloudy gray. The maternal surface was covered with blood clot and the fetal surface was distorted by several cystic spaces filled with gelatinous pink, translucent material. Early acute chorionitis, small placental infarcts, and fibrin pools were present microscopically.

Comment

When idiopathic inflammatory bowel disease is active and severe at the time of conception, spontaneous abortion, stillbirth, or premature delivery are more likely.[1] Sulfasalazine and steroids should be used for idiopathic inflammatory bowel disease in the pregnant woman

From the Departments of Obstetrics and Gynecology and Pathology, Truman Medical Center, affiliated teaching hospital of the University of Missouri-Kansas City.
Received for publication December 12, 1990; accepted May 1, 1990.
Reprint requests: Ila M. Peterson, MD, Truman Medical Center, 2301 Holmes, Kansas City, MO 64108.
6/1/22087

much as they are in the nonpregnant woman.[2] There is no firm evidence that use of sulfasalazine has any harmful effects on the fetus. However, it is advisable to maintain the daily dose of prednisone below 20 mg/day when possible because some reports have suggested an increase in stillbirths, reduction in birth weight, cleft palate, harelip, and finger anomalies associated with higher doses.[2] In this case the patient was steroid dependent and experienced severe exacerbation of her disease when steroids were withdrawn. The other effects of steroids are well documented: impaired inflammatory response, delayed wound healing, and muscle atrophy. These effects could prevent uterine sections from properly healing and thereby render the uterine wall incompetent. Dense scar could not be demonstrated in multiple sections of the ruptured uterus in this case. Previous cesarean section, both transverse and vertical, abruptio placentae, grand multiparity, previous curettage, manual removal of placenta or obstructed labor, oxytocin, epidural anesthesia, puerperal infection, and diethylstilbesterol exposure are factors known to predispose women to uterine rupture. In patients with uterine dehiscence (separation of uterine scar without rupture of the membranes), perinatal outcome is excellent. The number of previous cesarean sections, interval after previous cesarean section, placental localization, or ultrasonographic assessment do not predict the likelihood of uterine rupture for any one patient.

REFERENCES

1. Miller JP. Inflammatory bowel disease in pregnancy: a review. J R Soc Med 1986;79:221-5.
2. Atlaly RD, Weekes ARL. The treatment of gastrointestinal disease in pregnancy. Clin Obstet Gynecol 1986;13:335-47.

Maternal serum Down syndrome screening: Free β-protein is a more effective marker than human chorionic gonadotropin

James N. Macri, PhD,[a] Ramana V. Kasturi, BE, MS,[a] David A. Krantz, BS,[a] Edward J. Cook, PhD,[a] Norman D. Moore,[a] Jean A. Young,[a] Kathleen Romero, BS,[a] and John W. Larsen, Jr., MD[b]

Carle Place, New York, and Washington, D.C.

The use of quantitative human chorionic gonadotropin measurement in obstetrics has a long and successful history. Prior studies on the utility of human chorionic gonadotropin in Down syndrome screening have utilized assays that measure the intact human chorionic gonadotropin molecule. This study targeted a distinct marker, the human chorionic gonadotropin free β-protein, which is present in second-trimester maternal serum at much lower concentrations than is intact human chorionic gonadotropin. Our study of 29 cases of trisomy 21 and 450 control samples shows 80% detection efficiency with maternal serum α-fetoprotein, the free β-protein, and maternal age in pregnancies under 17 weeks' gestation. We conclude that the combination of maternal serum α-fetoprotein and the human chorionic gonadotropin free β-protein will be useful in the prenatal detection of trisomy 21. (Am J Obstet Gynecol 1990;163:1248-53.)

Key words: Screening, Down syndrome, free β-protein

The measurement of human chorionic gonadotropin (hCG) in maternal serum is under investigation as a biochemical marker for pregnancies at increased risk for trisomy 21. Human chorionic gonadotropin (hCG) is a complex glycoprotein produced by the placenta shortly after implantation of the fertilized ovum into the uterine wall. The intact hCG molecule is a heterodimer, consisting of two noncovalently bound subunits designated as α-subunit and β-subunit (Fig. 1). The free α- and β-proteins are produced within the syncytiotrophoblast, and are known to exist in maternal serum. Free α is coded for by the same gene that produces α-subunits of luteinizing human hormone, follicle-stimulating hormone, and thyroid-stimulating hormone. The β-proteins in all of these hormones are

From the Research Division, NTD Laboratories, Inc., Carle Place,[a] and the Department of Obstetrics and Gynecology, George Washington University Medical Center.[b]
Received for publication March 8, 1990; revised June 14, 1990; accepted June 18, 1990.
Reprint requests: James N. Macri, PhD, Research Division, NTD Laboratories, Inc., 383 Old Country Road, Carle Place, NY 11514.
6/1/23215

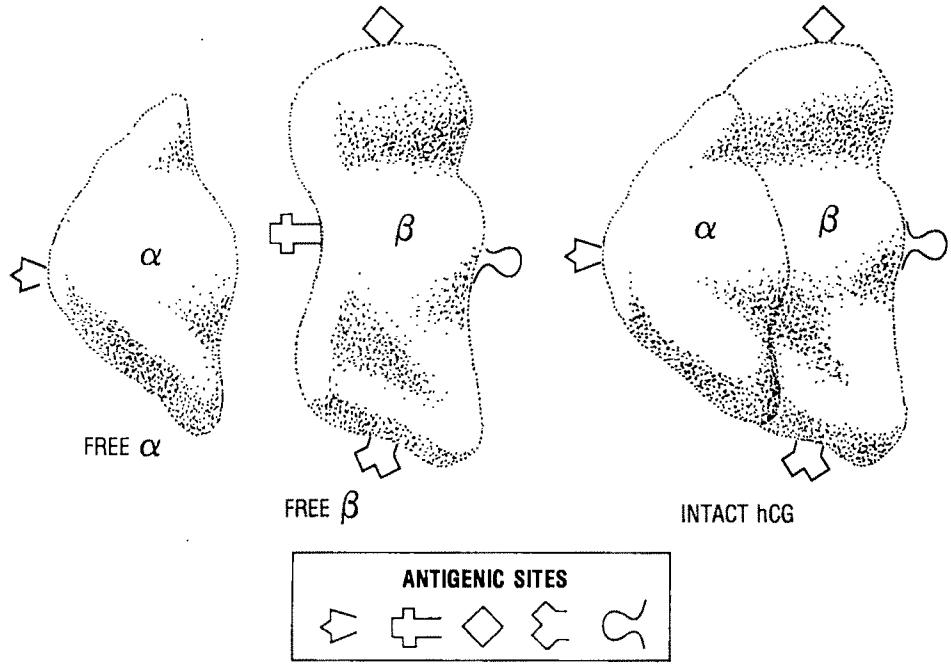


Fig. 1. Schematic representation of hCG molecule and its subunits.

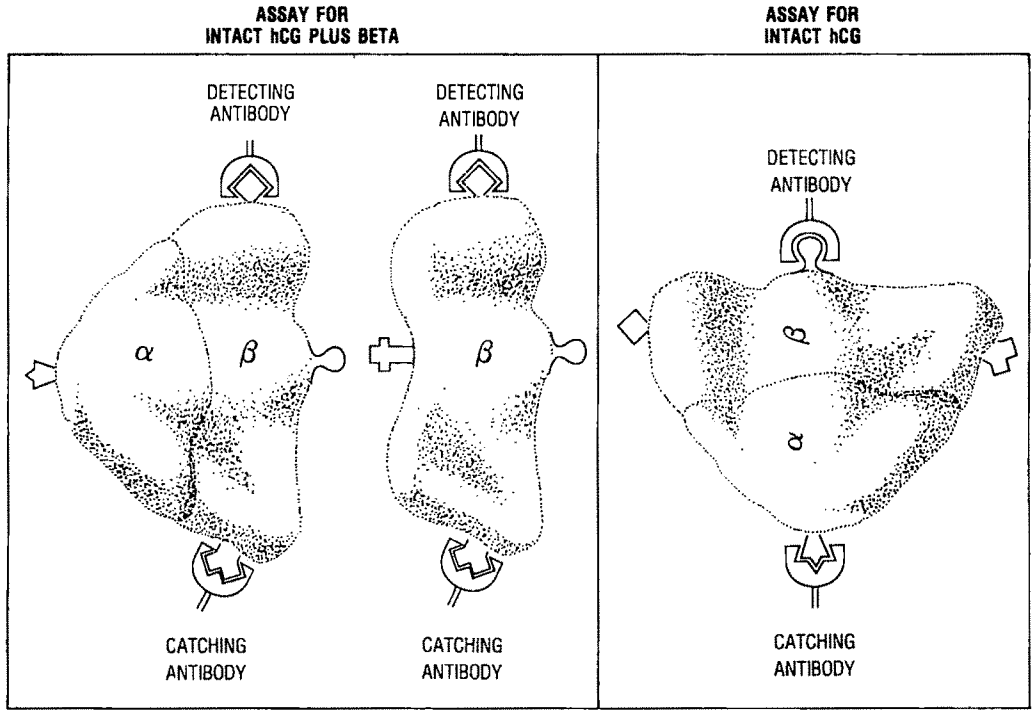


Fig. 2. Illustration of two-site immunoassays designed to measure (1) intact hCG plus free β-protein and (2) intact hCG alone.

coded for by distinct genes and display differing immunologic characteristics. In normal second-trimester maternal sera, intact hCG is found in levels ranging from 20,000 mIU/ml to 50,000 mIU/ml. In contrast, the levels of either free α- or free β-hCG are on average one half of 1% of intact hCG levels.[1]

Two methods are commonly used in measuring hCG (Fig. 2). The first measures intact hCG alone; the sec-

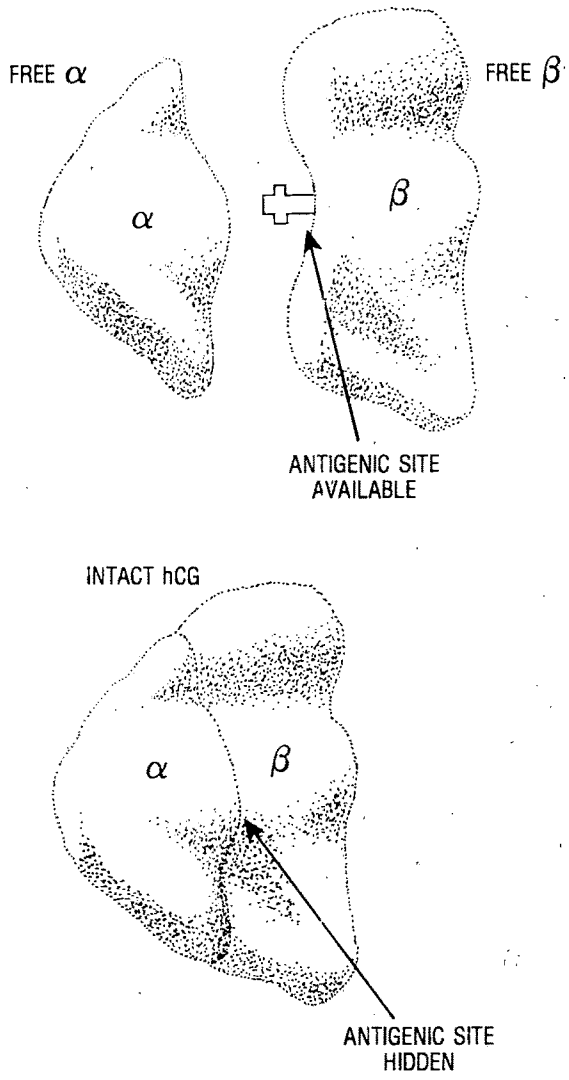


Fig. 3. Schematic representation of free β-protein antigenic site. Note its unavailability in intact hCG molecule.

ond measures intact hCG plus free β-protein. These methods are considered to be equivalent because the amount of free β-protein is quantitatively insignificant in comparison with intact hCG. However, in our laboratory, both of these assay methods, when investigated as to their potential to detect Down syndrome, yielded dissimilar results. This observation as well as the fact that free β is unique to hCG, led us to hypothesize that an assay directed specifically against the free β-protein might be efficient for Down syndrome screening.

Material and methods

Four hundred seventy-nine maternal sera from 29 cases of trisomy 21 and 450 controls were retrieved from frozen storage at NTD Laboratories. These samples were from singleton, nondiabetic, white gravid women. All outcome information on these samples was obtained via requests to physicians.

Table I. Median maternal serum free β-protein levels

Weeks' gestation	*Control (ng/ml)*	*Trisomy 21 (ng/ml)*
14-16	16.3	34.7
17-18	10.9	19.9

Table II. Maternal serum free β-protein levels in terms of multiples of median in 29 confirmed trisomy 21 cases

Multiples of median	*No.*	*% of Total*
0-1	5	17.2
1-2	9	31.0
2-3	5	17.2
>3	10	34.5

Assays. Maternal serum α-fetoprotein (AFP) was quantitated by an in-house enzyme-linked immunosorbent assay (ELISA). This ELISA is a polyclonal-monoclonal assay with an effective working range of 2 to 250 IU/ml of AFP. Standards were calibrated against the World Health Organization standard 72/225.

Free β-protein was quantitated by an in-house ELISA that is a monoclonal-polyclonal assay with an effective working range of 1.5 to 100 ng/ml of free β-protein. Interassay and intraassay variation (on trilevel quality control samples run 236 times) was found to be 4.3% and 5.8%, respectively. Standards were calibrated against the first international reference preparation (IRP 75/551).

In assaying for free β-protein (Fig. 3), antibodies must be directed specifically against an antigenic site that is exposed only when free beta exists in its unbound form (i.e., not combined with the α-subunit to form the intact hCG molecule). The specific free β-antibody used in our assay procedure does not react with any other antigenic site on the β-subunit of intact hCG or any antigenic site on the α-subunit. In addition, the assay procedure used in this study ensured that only free β was measured by introducing a thorough washing step after the initial capture of free β-protein. The washing step effectively removes any analyte not initially captured by the specific free β-antibody. Such a procedure removes from the assay the intact hCG molecules that predominate in second-trimester maternal sera.

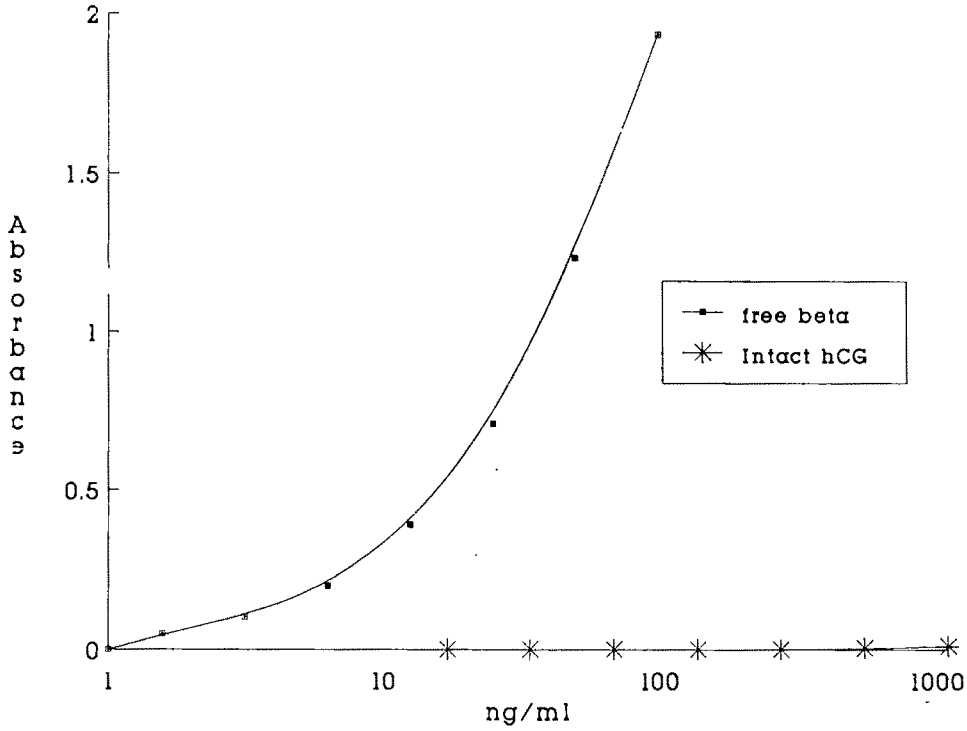


Fig. 4. Free β-protein assay absorbance values obtained with: (1) free β-protein standards and (2) serially diluted intact hCG standard from which free β-protein has been removed. Absence of cross-reactivity to intact hCG is demonstrated.

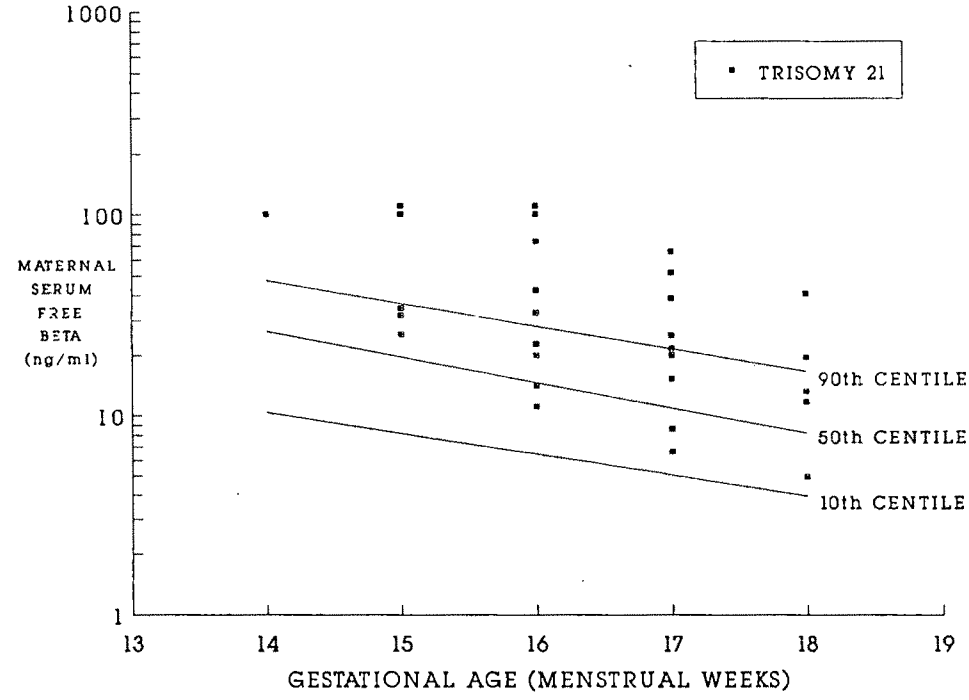


Fig. 5. Levels of free β-protein in cases of trisomy 21 in relation to 10th, 50th, and 90th percentiles of controls.

Table III. Trisomy 21 screening efficiency with free β-protein hCG alone and in combination with AFP at different gestational age ranges at 5% false-positive rate

Gestational age range (wk)	*No. of controls*	*No. of cases of trisomy 21*	*Detection efficiency of free β-protein (%)*	*Detection efficiency of AFP and free β-protein (%)*
<17	240	15	66.7	80
≥17	210	14	50.0	64.3
14-22	450	29	58.6	72.4

Proof of antigenic specificity to free β-protein was obtained by passing an intact hCG sample containing 1000 ng/ml over a free β-protein affinity chromatography column to effectively remove free β-protein. This sample (now void of free β-protein) was evaluated for cross-reactivity to intact hCG in our free β-protein assay. Fig. 4 demonstrates essentially no cross reactivity. This provides proof that our free β-antibody (and assay) does not react with epitopic sites on intact hCG but does react with epitopes that are exposed when free β-protein exists in its unbound state.

To determine detection efficiency and false-positive rates we used a linear, multivariate, discriminant analysis model. Gestational age was included as a variable in the model along with biochemical markers. The natural logarithm of the level of each analyte was used. Linear, multivariate, discriminant analysis was performed with the commercially available software package SAS (Statistical Analysis System, SAS Institute, Inc., Cary, N.C.). Population parameters (means, variances, and covariances) for each of the biochemical markers and gestational age were established for the cases of trisomy 21 and controls. The variances and covariances were pooled among the trisomy 21 cases and controls. Each patient's risk of trisomy 21 was calculated from Bayes' rule and the patient's maternal age-specific a priori risk.[2]

Results

As shown in Table I, a significant increase in the level of free β-protein was observed in trisomy 21 cases as compared with controls ($p < 0.0001$ using the Wilcoxon test). Fig. 5 illustrates the downward trend of free β-protein levels in controls by gestational age. Table II shows the distribution of the 29 trisomy 21 cases and reveals that 83% exceed the median of controls, and 52% exceed twice the median, for their respective gestational weeks.

Detection efficiencies for free β-protein alone and in combination with AFP are shown in Table III. Maximal detection efficiency was achieved by using maternal serum AFP and free β-protein at 14 to 16 weeks.

There was no trend in either maternal serum AFP or free β-protein levels with increasing maternal age for trisomy 21 cases or controls ($p = 0.24$ and 0.28 for maternal serum AFP, and $p = 0.85$ and 0.10 for free β-protein using Kruskal-Wallis analysis of variance for maternal serum AFP and free β-protein, respectively, across four different maternal age groups ≤26, 27 to 28, 29 to 31, and >31 years). Additionally, the correlation of the levels of free β-protein and maternal serum AFP was not significantly different from zero ($R = 0.11$, $p = 0.57$, and $R = -0.05$, $p = 0.28$ for trisomy 21 cases and controls, respectively).

Comment

The use of quantitative hCG measurement as a pregnancy test has had a long, straightforward, and successful history. However, use of commercially available hCG kits for prenatal Down syndrome screening is a more complex matter. Commercial kits that measure the intact hCG molecule plus free β-protein are often referred to as β-subunit hCG kits.[3] Superior detection efficiency in our study was achieved, not by measuring intact hCG in a so-called β-subunit hCG kit, but in fact by eliminating the intact hCG molecule through immunologic and assay washing procedures. Intact hCG plays no immunologic (and, by definition, no quantitative) role in what our assay measures. The analyte we measured is the free β-protein, which is a specific, independent marker present in second-trimester maternal sera at much lower concentrations than are intact hCG molecules (on average 1:200).

Our data show that quantitation of maternal serum free β has high predictive value (50% to 67%) for the serologic detection of trisomy 21. In fact, the use of an assay that measured only free β-protein produced a detection efficiency and false-positive rate comparable to that reported by some others using a combination of three assays.[4] In our study the highest rate of detection efficiency (80%) was achieved by combining analyses of free β-protein with maternal serum AFP. This combination appears to work optimally during weeks 14 through 16 of gestation, producing a detection efficiency of 80% with a 5% false-positive rate. Evaluation at even earlier weeks of gestation, when free β-protein levels are higher,[5] should be performed to determine whether further enhancement in detection efficiency results from such evaluation.

Whereas Bogart et al.[6] and Wald et al.[4] observed the

effectiveness of intact hCG as a marker for Down syndrome, the effectiveness of the free β-protein was not observed. By using assays specific to free β-protein, the analyte can be evaluated for its usefulness in Down syndrome screening. This was done in this investigation.

Conventional hCG assays that measure the intact hCG molecule require significant dilution of the sample (1/50 to 1/500) to bring the level of the analyte to a reasonable analytical range for measurement. There is no need for a predilution step in free β-protein assays. This represents an additional advantage from the perspective of assay development and use, as well as minimization of human error.

Our findings show that it is effective to include a combination of maternal serum AFP and free β-protein assays in a screening protocol for trisomy 21. The advantages of such a protocol include the following: (1) noninvasive techniques, (2) high detection efficiency with low false-positive rates, (3) simplified, undiluted free β-protein assays, (4) the use of markers that are essentially independent of one another, and (5) compatibility with current prenatal screening protocols.

REFERENCES

1. Ozturk M, Berkowitz R, Goldstein D, Bellet D, Wands JR. Differential production of human chorionic gonadotropin and free subunits in gestational trophoblastic disease. AM J OBSTET GYNECOL 1988;158:193-8.
2. Hook EB, Chambers GM. Numerical taxonome on birth defects and polygenic disorders. In: Bergsma D, Lowry RB, Trimble BK, Feingold M, eds. Birth defects original article series. New York: Alan R Liss, 1977 vol 13 no 3A:123-41.
3. Aakvaag A, Leskinen E, Lindstedt G, Moller J, Byberg A, Weber T. Kits for the quantitative assay of chorionic gonadotropin. Scand J Clin Lab Invest 1988;48:299-302.
4. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. Br Med J 1988;297:883-7.
5. Hay DL. Placental histology and the production of human choriogonadotropin and its subunits in pregnancy. Br J Obstet Gynaecol 1988;95:1268-75.
6. Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn 1987;7:623-30.

Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation from amniotic fluid

Carl P. Weiner, MD, and Charles Grose, MD

Iowa City, Iowa

Cytomegalovirus was isolated antenatally from the amniotic fluid of two pregnant women. In both cases prenatal diagnosis of the fetal virus infection aided the subsequent management of the mother and newborn infant. (AM J OBSTET GYNECOL 1990;163:1253-5.)

Key words: Cytomegalovirus, amniocentesis, prenatal diagnosis, congenital infection

In 1971 Davis et al.[1] published the first case of fetal cytomegalovirus infection diagnosed prenatally by isolation of the virus from amniotic fluid. The case report concerned a 17 year-old primigravid woman who was seen with a "mononucleosis-like syndrome" (fever, splenomegaly, thrombocytopenia, lymphocytosis) at 21 weeks' gestation. Amniotic fluid was aspirated by transabdominal amniocentesis, and cytomegalovirus was grown readily from the amniotic fluid. Since their report there has been some controversy concerning the use of amniotic fluid culture for the diagnosis of cytomegalovirus. We describe two additional cases of fetal cytomegalovirus infection diagnosed antenatally by isolation of the virus from amniotic fluid.

From the Departments of Obstetrics and Gynecology and Pediatrics, University of Iowa College of Medicine.
Supported by grants from the National Institutes of Health.
Received for publication April 4, 1990; accepted April 4, 1990.
Reprint requests: Charles Grose, MD, Department of Pediatrics, University of Iowa Hospitals, Iowa City, IA 52242.
6/1/21410

Case reports

Case 1. The patient was a 17-year-old white primigravid woman transferred to the University Hospital at 37 weeks' gestation. The pregnancy had been uncomplicated until the day before transfer when the patient had malaise, bilateral lower abdominal discomfort,

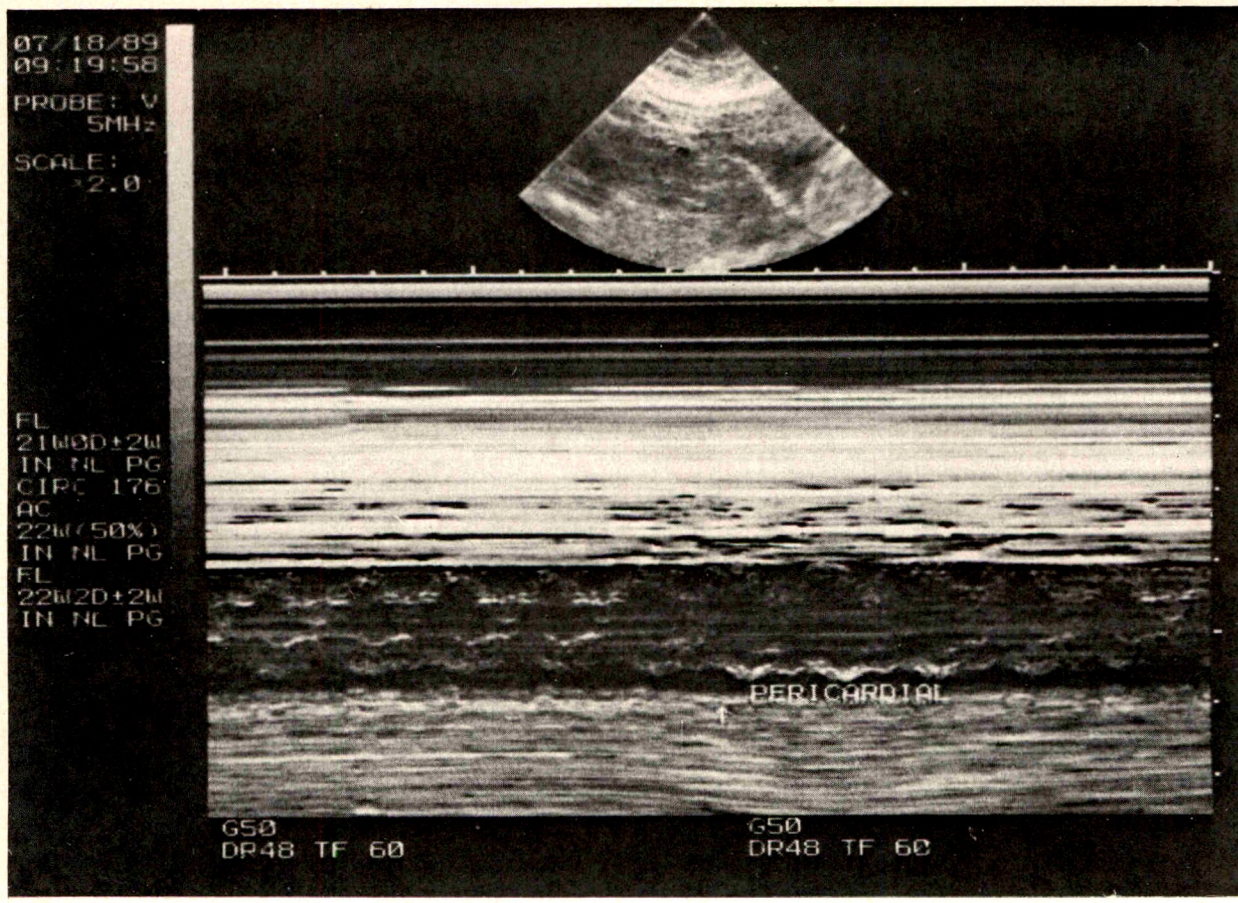


Fig. 1. M-mode echocardiogram of fetus in case 2. Pericardial effusion *(small arrow)* was detected below left atrium.

loose stools, and fever. Her symptoms were not associated with dysuria, vaginal discharge, or leakage of amniotic fluid. The physical examination revealed an uncomfortable, diaphoretic pregnant woman. Vital signs were normal except for fever of 38.8° C. Both urinalysis and culture results were negative. The uterus was diffusely tender to palpation. Real-time ultrasonography of the uterus demonstrated severe oligohydramnios and a viable fetus with right hydronephrosis and biometric criteria suggestive of growth retardation. Because the differential diagnosis included amnionitis, a transabdominal amniocentesis was performed with aspiration of 17 ml of clear amniotic fluid. Both Gram stain and bacterial culture of amniotic fluid were negative. However, viral culture of the amniotic fluid was positive within 72 hours. The identity of the viral isolate as cytomegalovirus was confirmed by a cytomegalovirus-specific monoclonal antibody. Serologic testing for specific maternal antibodies to cytomegalovirus revealed no IgM response (0.10 enzyme-linked immunosorbent assay units; negative), but a cytomegalovirus-specific IgG titer was positive at 0.50 enzyme-linked immunosorbent assay units (mid-positive).

Labor was induced at 38 weeks' gestation to facilitate treatment if required. The patient was delivered vaginally of a 2470 gm male with Apgar scores of 7 and 9 at 1 and 5 minutes, respectively. Physical examination revealed an active newborn without stigmata of congenital cytomegalovirus infection. An ultrasound examination confirmed the presence of unilateral right hydronephrosis. Although an ultrasonogram of the brain on day 1 revealed a normal ventricular system without calcifications, a second study done 1 week later showed a developing cystic formation in the caudate nucleus. The cerebrospinal fluid had an elevated cell count (2281 monocytes/mm^3) and elevated protein (100 mg/dl). Bacterial cultures were negative. A virology culture of the cerebrospinal fluid was not obtained. Nevertheless, these results are suggestive of continued postnatal viral disease in the central nervous system. Viral cultures of the infant's throat secretions and urine on day 2 of life both grew cytomegalovirus.

Case 2. The patient was a 25-year-old white woman (gravida 3, para 1) referred to the University of Iowa Fetal Diagnosis and Treatment Unit at 23 weeks, after routine ultrasonography demonstrated what was thought to be a left pleural effusion. The mother denied any recognizable illness during the gestation. When a level II ultrasonographic examination was con-

ducted, the effusion was noted to be pericardial rather than pleural (Fig. 1). An intermittent bradyarrhythmia also was documented. Amniotic fluid volume and fetal biometric parameters were within normal range.

Amniocentesis was performed to determine fetal karyotype and obtain viral cultures. Maternal serum was drawn on the same visit. It was positive for both cytomegalovirus-specific IgG (1.77 ELISA units; high positive) and cytomegalovirus-specific IgM (0.35 ELISA units; low positive). The amniotic fluid culture grew cytomegalovirus. Chromosomal analysis revealed a normal 46,XY karyotype. A third ultrasonographic examination 1 week after the second demonstrated complete resolution of the pericardial effusion. The fetus continued to grow appropriately for the duration of the gestation and weighed 3440 gm when delivered spontaneously at term. Physical examination of the newborn revealed no signs of congenital cytomegalovirus infection.

Comment

Cytomegalovirus, a member of the family *Herpesviridae*, is the most common cause of congenital infection.[2] The mechanism of cytomegalovirus transmission to the fetal tissues remains to be elucidated but is thought to occur hematogenously. Because the kidney is an organ that is commonly involved during cytomegalovirus infection, the infected fetus presumably excretes cytomegalovirus via fetal urine into the amniotic fluid. In addition to our two cases, there have been eight previous case reports of antenatally diagnosed cytomegalovirus infection after amniocentesis.[2] With this increasing number of case reports, we suggest that cytomegalovirus culture of amniotic fluid be considered in pregnant women in whom primary cytomegalovirus infection is suspected (e.g., in symptomatic pregnant adolescents, as in our first case). Similarly, cytomegalovirus cultures of amniotic fluid may be indicated when second-trimester ultrasonography reveals fetal problems compatible with acute viral infection, as in our second case.

REFERENCES

1. Davis LE, Tweed GV, Chin TDY, Miller GL. Intrauterine diagnosis of cytomegalovirus infection: viral recovery from amniocentesis fluid. AM J OBSTET GYNECOL 1971;109: 1217-9.
2. Grose C, Itani O, Weiner CP. Prenatal diagnosis of fetal infection: advances from amniocentesis to cordocentesis—congenital toxoplasmosis, rubella, cytomegalovirus, varicella virus, parvovirus and human immunodeficiency virus. Pediatr Infect Dis J 1989;8:459-68.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Pelvimetry by magnetic resonance imaging in breech presentation

Aren J. van Loon, MD,[a] Albert Mantingh, MD, PhD,[a] Cornelis J. P. Thijn, MD, PhD,[b] and Eduard L. Mooyaart, MD, PhD[b]

Groningen, The Netherlands

Evaluation of the pelvis by pelvimetry plays an important role in selecting patients with term breech presentation for possible vaginal delivery. X-ray pelvimetry involves the disadvantage of fetal exposure to ionizing radiation. Computed tomography reduces the amount of radiation markedly, but there is still a risk. Ten patients with breech presentation at term underwent antepartum pelvimetry with magnetic resonance imaging, followed by postpartum magnetic resonance pelvimetry and x-ray pelvimetry. Measurements with magnetic resonance imaging are as reliable as those obtained with x-ray pelvimetry and the contrast of the magnetic resonance images is even better. The advantages of magnetic resonance pelvimetry are clear: no ionizing radiation and reliable pelvimetry results in cases of term breech presentation in which a normal pelvis is a prerequisite for safe vaginal delivery. (AM J OBSTET GYNECOL 1990;163:1256-60.)

Key words: Magnetic resonance imaging, breech presentation, pelvimetry

Controversy continues to exist regarding the value of pelvimetry.[1-3] It should be emphasized that a distinction must be made between pelvimetry in vertex presentation and in breech presentation. The value of pelvimetry in vertex presentation is doubtful; usually trial of labor can be allowed in all cases provided that high and midforceps deliveries are abandoned ("the fetal head is the best pelvimeter").

In breech presentation the value of pelvimetry is clear, provided that other parameters are considered in addition to the result of pelvimetry when deciding whether a term breech presentation can be delivered vaginally.[4-11] The disadvantage of x-ray pelvimetry is clear: ionizing radiation.[12] With computed tomography the radiation dose is reduced by approximately 65% but a certain radiation load still exists.[13, 14] Ultrasonographic pelvimetry is often unreliable and the pelvic configuration cannot be established properly. With magnetic resonance imaging (MRI) a new method for pelvimetry has become available. This technique makes imaging in multiple planes possible and combines the advantages of conventional or computed tomographic pelvimetry with the benefits of ultrasonographic techniques (no ionizing radiation).[15, 16] To evaluate the reliability of this relatively new method for pelvimetry, antepartum MRI was performed in 10 patients with breech presentation at term. This was followed by postpartum pelvimetry with MRI and conventional x-rays. The results of all three investigations were compared. To our knowledge this is the first report of magnetic resonance pelvimetry compared with conventional x-ray pelvimetry and antepartum pelvimetry compared with postpartum pelvimetry.

From the Departments of Obstetrics and Gynecology[a] and Radiology,[b] University Hospital.
Received for publication November 28, 1989; revised April 9, 1990; accepted June 2, 1990.
Reprint requests: Aren J. van Loon, MD, Department of Obstetrics and Gynaecology, University Hospital Groningen, 59 Oostersingel, 9713 EZ Groningen, The Netherlands.
6/1/22870

Material and methods

From April 1987 to May 1988 MRI pelvimetry was performed in 10 patients near or at term (36 to 41 weeks). The actual time was determined by the time of referral because of breech presentation and by the availability of the MRI equipment. Patients were informed about the study and its procedure by their own physicians, who were not involved in the study. It was explicitly explained that participation or refusal would not influence clinical management. Patients were informed that the results of the pelvimetry would have no influence on the decision about the mode of delivery. (Only postpartum conventional x-ray pelvimetry in selected cases is performed in The Netherlands.) Oral informed consent was obtained from all patients who agreed to participate. The study was approved by the Institutional Medical Ethical Committee. In the first week post partum (in two patients several weeks later) pelvimetry was repeated with MRI and conventional x-rays. The MRI pelvimetry was repeated to exclude

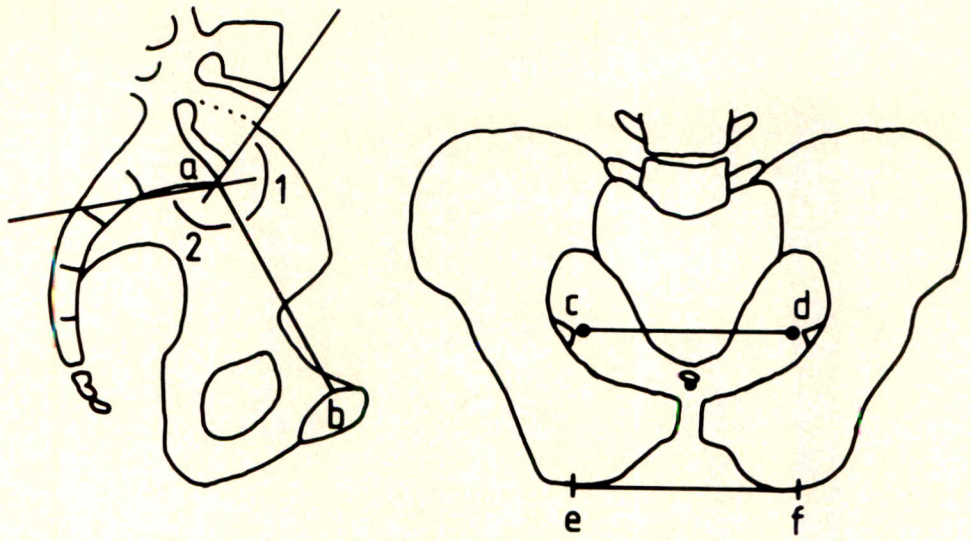


Fig. 1. Pelvic distances and angles *a-b*, Obstetric conjugate (≥11.5 cm); *1*, pelvic inlet angle (≥115 or ≤150 degrees); *2*, pelvic aperture angle (≥80 or ≤120 degrees); *c-d*, interspinal distance (≥9.5 cm); *e-f*, intertuberal distance (≥10.5 cm). Values given are considered critical diameters and angles for allowing vaginal delivery of a term breech.

differences between the antepartum and postpartum situations. On the lateral or sagittal view the obstetric conjugate, the angles formed by the obstetric conjugate, the lumbar and sacral spine, respectively, were measured and we described the configuration of the pelvis. The purpose of measuring the pelvic inlet angle formed by the obstetric conjugate and the lumbar spine, and the pelvic aperture angle, formed by the obstetric conjugate and the sacral spine, is to obtain information about the axis and the configuration of the pelvic cylinder. Abnormal configuration might cause the breech to be caught on the symphysis, or cause rotation problems of the unmolded aftercoming fetal head.[17] On the anteroposterior or coronal view the interspinal and the intertuberal distances were measured. The minimal acceptable pelvimetry data for vaginal delivery of a term breech were derived from previous studies.[3, 17] The pelvic distances and angles with critical values are shown in Fig. 1.

The magnetic resonance images were obtained with a 1.5 tesla magnet system (Philips, Eindhoven, The Netherlands; Gyroscan). Sagittal (Fig. 2) and coronal (Fig. 3) slices 10 mm thick were made with a repetition time of 650 msec and an echo time of 20 msec. Data were acquired with a 256 × 180 matrix. The x-ray images were obtained in accordance with Guthman's method (one-shot lateral pelvimetry, patient in lateral position with hips and knees in slight flexion and a lead rod, with centimeter markings, between the legs; cen-

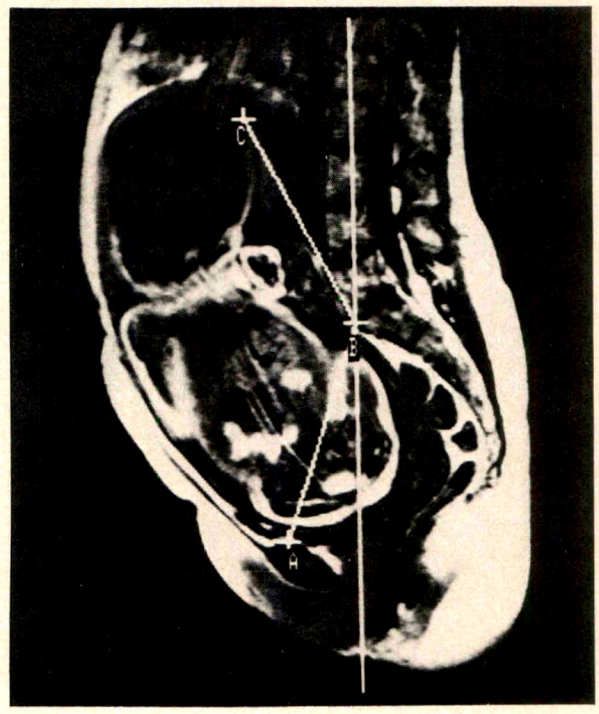


Fig. 2. Sagittal, midline MRI of fetus in breech presentation. Maternal pelvis is of normal size and configuration. Electronic cursor measures distance between symphysis pubis *(A)* and sacral promontory *(B)* (anteroposterior pelvic inlet diameter or obstetric conjugate). The angle formed by the line *A-B-C* is the upper pelvic inlet angle, formed by obstetric conjugate and lumbar spine.

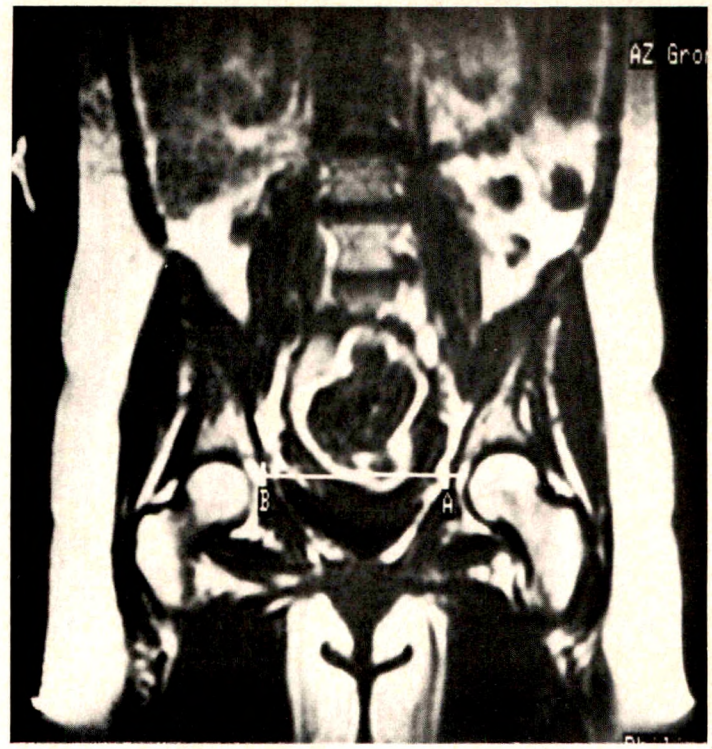


Fig. 3. Coronal MRI. Interspinal distance is measured (*A-B*).

Table I. Means of pelvic dimensions and angles, including SD and significance levels of differences between means*

	Obstetric conjugate (cm)	*Interspinal distance (cm)*	*Intertuberal distance (cm)*	*Pelvic inlet angle (degrees)*	*Pelvic aperture angle (degrees)*
MRI-1	14.08 ± 0.66	11.27 ± 0.45	11.04 ± 0.99	137 ± 15	91.8 ± 13
MRI-2	13.64 ± 0.46	11.14 ± 0.42	10.97 ± 0.87	136.8 ± 15	90.3 ± 14
X-ray	13.42 ± 0.75	10.62 ± 0.50	11.84 ± 0.87	134 ± 15.5	91.5 ± 13.5
Combination A	0.05	—	—	—	—
Combination B	0.05	0.01	0.01	—	—
Combination C	—	0.01	0.05	—	—

Pelvic inlet angle: Obstetric conjugate − Lumbar spine; Pelvic aperture angle: Obstetric conjugate − Sacral spine.

MRI-1, Antepartum; *MRI-2,* postpartum; *x-ray,* postpartum; *Combination A,* MRI-1 − MRI-2; *Combination B,* MRI-1 − x-ray; *Combination C,* MRI-2 − x-ray.

*Wilcoxon rank-sum test for paired samples.

tration on femoral head), and a second radiograph was taken in anteroposterior direction. In every patient ultrasonographic examination was done to estimate the fetal body weight and the biparietal diameter and flexion of the fetal head and to determine the placenta localization. The duration of magnetic resonance pelvimetry was approximately 15 minutes; duration of x-ray pelvimetry was about 10 minutes.

Of the original group of 12 patients, two were excluded from further participation because of extreme claustrophobia during postpartum magnetic resonance pelvimetry. Statistical analysis of all the results was performed with the Wilcoxon rank-sum test for paired samples.

Results

The mean values (including SDs) of the obstetric conjugate, interspinal distance, intertuberal distance, pelvic inlet angle, and pelvic aperture angle and the levels of significance are shown in Table I. No differences in the general description of the configuration of the pelvis were observed. In Table II the data for all patients are shown. The antepartum magnetic resonance pelvimetry was used for the pelvimetry description. Only one of the 10 patients was delivered vaginally. Four cesarean sections were performed because of lack of progress during labor. Two of these patients had an abnormal pelvis; pelvimetry of patients no. 1 and no. 9 showed an abnormal configuration (Fig. 4) and a

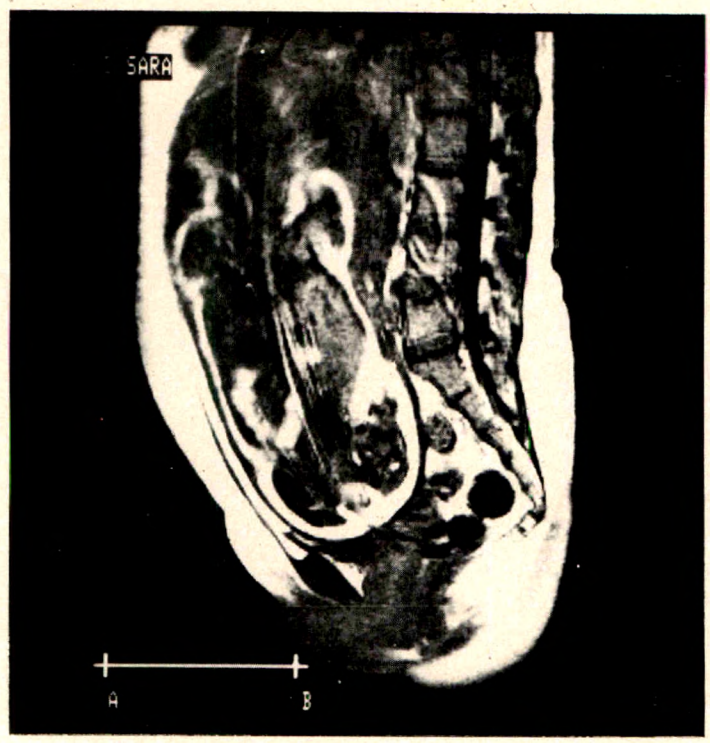


Fig. 4. Sagittal, midline MRI of patient 1. Abnormal configuration is seen; sacrum is not hollow and os coccygis forms an angle of almost 90 degrees with last sacral spine.

Table II. Results per patient

Patient No.	*Pelvimetry*	*Manual assessment*	*Planning*	*Result*	*Birth weight (gm)*
1	Abnormal	Normal	Trial of labor	CS*	3410
2	Normal	Normal	Trial of labor	Vaginal delivery	2900
3	Abnormal	Abnormal	CS	CS	3060
4	Normal	Normal	Trial of labor	CS*	3190
5	Normal	Normal	CS†	CS	3390
6	Abnormal	Abnormal	CS	CS	3300
7	Normal	Normal	CS†	CS	3920
8	Abnormal	Abnormal	CS	CS	3080
9	Abnormal	Normal	Trial of labor	CS*	3570
10	Normal	Normal	Trial of labor	CS*	3860

CS, Elective cesarean section because of abnormal manual assessment.
*Cesarean section because of lack of progress during vaginal delivery.
†Elective cesarean section because of high presenting part.

small interspinal distance (<9.5 cm). Of the five patients who had elective cesarean sections, patients no. 5 and no. 7 had completely normal pelvimetry and manual pelvic assessment results. Three patients (nos. 3, 6 and 8) appeared to have abnormal pelvic angles.

Comment

The obstetric conjugate appears to be longer during pregnancy than post partum; this difference is significant at the 5% level. However, the clinical relevance is questionable and the only conclusion that can be drawn is that the generally acccepted minimum x-ray values might be too cautious. Differences were found between the interspinal and intertuberal distances on the magnetic resonance pelvimetries, on the one hand, and the x-ray pelvimetries, on the other, but not between the two magnetic resonance pelvimetries. The interspinal distance on the x-ray pelvimetry was smaller and the intertuberal distance was larger than on the magnetic resonance pelvimetry. An explanation for the differences might be that x-ray pelvimetry in the anteroposterior view is not always reliable. In many cases this x-ray image was not very clear. However, the differences were so small that it was considered acceptable to apply x-ray image values that are acceptable for vaginal delivery to the magnetic resonance images. Mag-

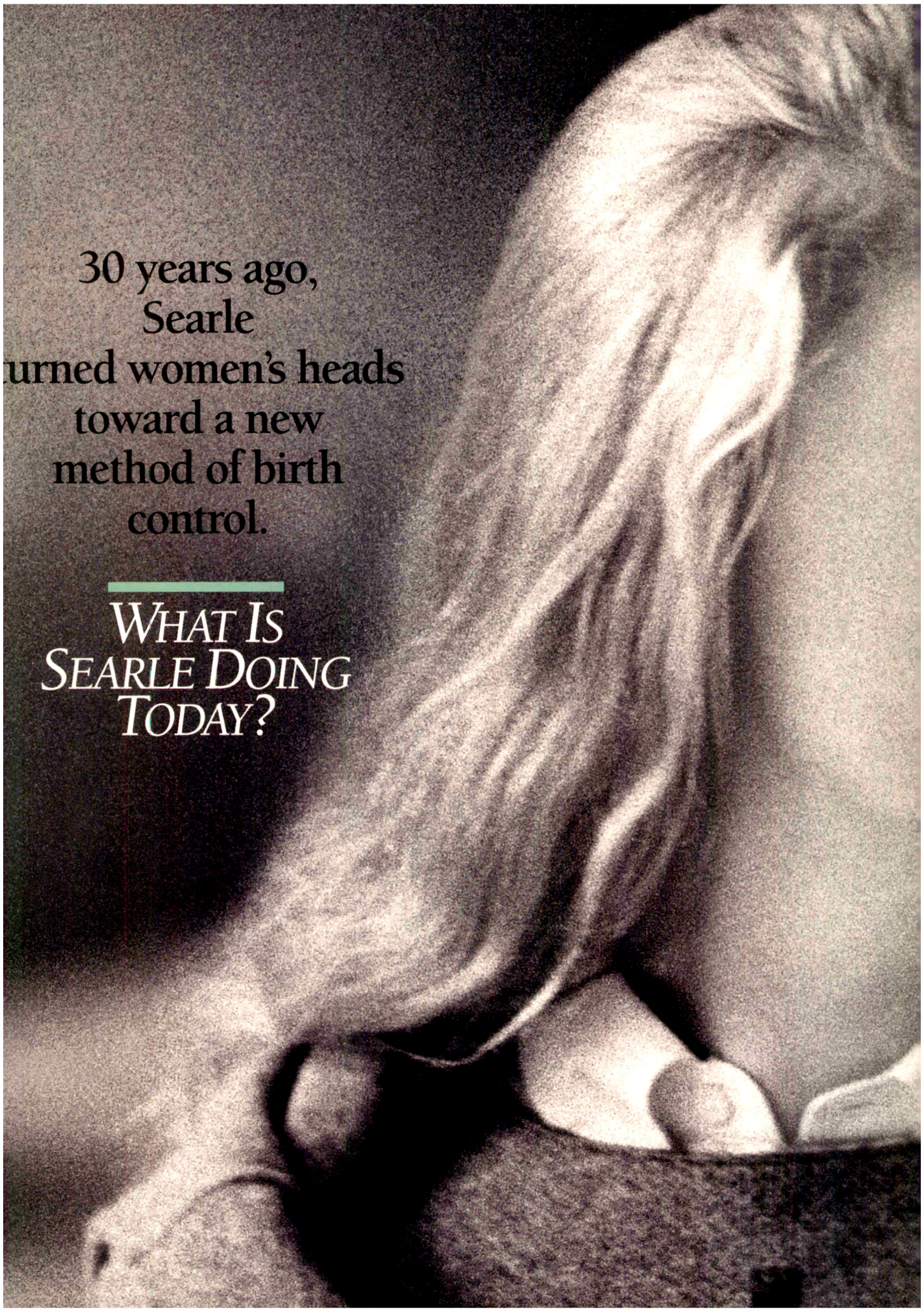
netic resonance pelvimetry was particularly suitable for assessment of the pelvis because there were no differences between the general descriptions, and the contrast of the magnetic resonance images was superior. After review of the results per patient it appears that elective cesarean section was performed in two cases (No. 5 and No. 7) with a normal pelvis (on manual and pelvimetry assessment) because of a high presenting part. With the knowledge obtained from magnetic resonance pelvimetry, vaginal delivery would have been acceptable. In two patients (No. 1 and No. 9) the pelvis was normal on manual assessment, whereas pelvimetry showed abnormal values. In these two patients vaginal delivery was thought to be acceptable but the pelvimetry results favored an elective cesarean section. As in patients 4 and 10 it is clear that even if the manual assessment and pelvimetry are normal, cesarean section can be inevitable because of lack of progress during vaginal delivery. Patients 3, 6, and 8 showed that the manual assessment was confirmed by magnetic resonance pelvimetry. We therefore conclude that although magnetic resonance pelvimetry results alone should never be the only parameter for accepting or rejecting vaginal delivery in breech presentation, a normal pelvis is the most important factor. This study shows that precise pelvimetry is possible with MRI and that a good general impression can be obtained of an abnormal configuration, even if the distances are normal. A manual assessment is insufficient: the negative predictive value in our group was only 71.5%. If an obstetrician decides that a cesarean section is indicated in any breech presentation at term, all types of pelvimetry will be useless. However, if vaginal delivery is thought to be acceptable under certain conditions, we believe that pelvimetry should be included in the evaluation of these conditions. Magnetic resonance pelvimetry may prevent unnecessary cesarean sections, it may prevent a wrongly accepted vaginal delivery, and it certainly prevents the fetus from being exposed to ionizing radiation. The contraindications to the use of this scanning technique are limited: only patients with artificial, metal-containing heart valves, pacemakers, and vascular metal clips, etc., have a contraindication. Extreme claustrophobia might appear to be a relative contraindication. There are no known or foreseeable hazards or risks for mother or fetus associated with MRI.[18]

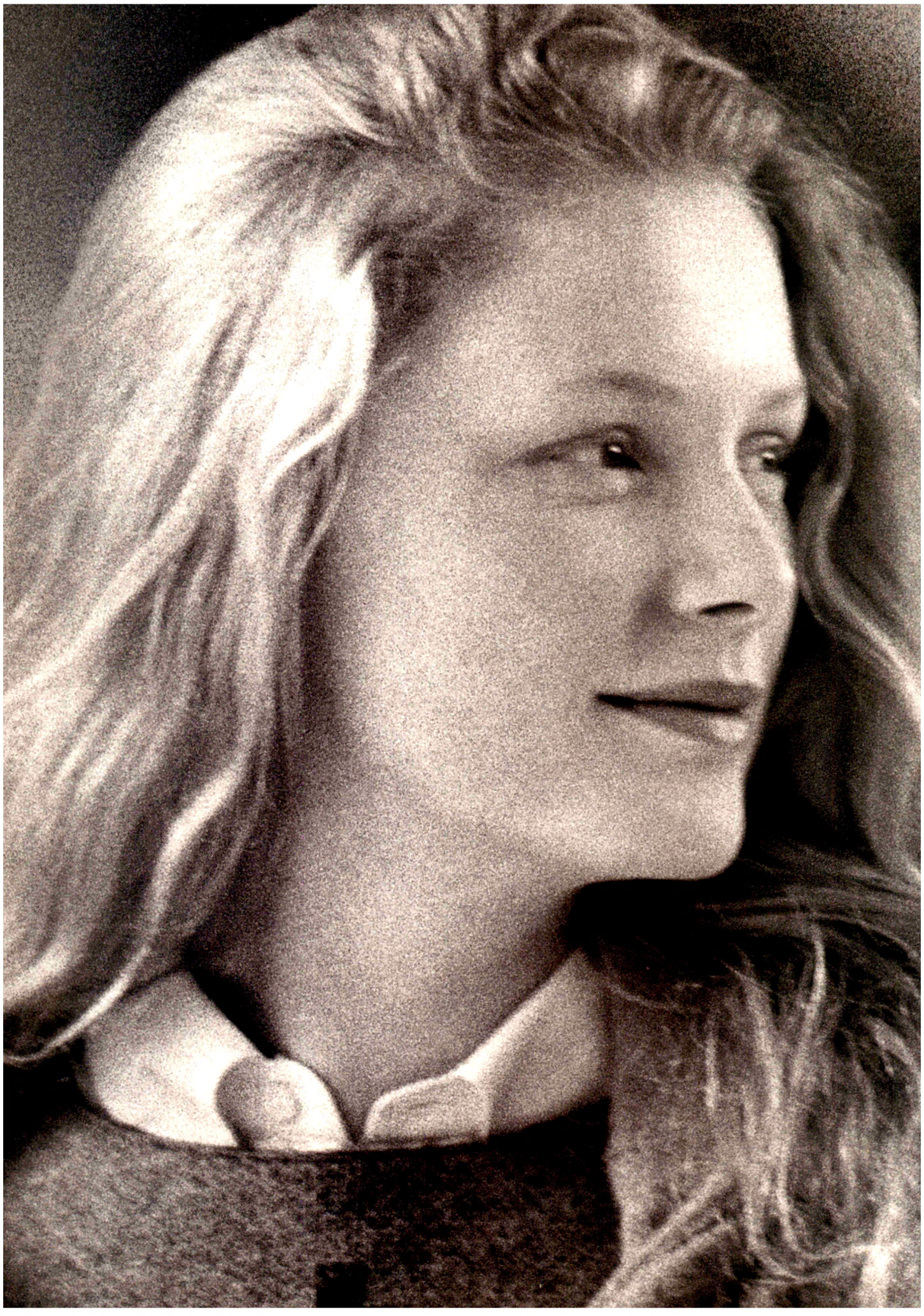
At present MRI is still relatively expensive compared with computed tomography and conventional x-ray pelvimetry (two and eight times, respectively) and is not yet available on a large scale. However, the costs of a MRI scan will soon be the same as a computed tomographic scan. With the arrival of new MRI systems, such as the Gyroscan T5, many hospitals will have an MRI system in the near future. These new systems are less expensive, easier to use, do not require special constructions, and might cause less claustrophobia because the design allows more space for the patient.

Our results indicate that magnetic resonance pelvimetry provides reliable information about the geometry of the pelvis without the use of ionizing radiation. The study shows that magnetic resonance pelvimetry is technically accurate and could now be used to assess the predictive value of this new diagnostic tool for a safe clinical management of breech presentation at term. A prospective study with a larger series of patients is required.

REFERENCES

1. Fine EA, Bracken M, Berkowitz RL. An evaluation of the usefulness of x-ray pelvimetry: comparison of the Thoms and modified Ball methods with manual pelvimetry. Am J Obstet Gynecol 1980;137:15-20.
2. Joyce DN, Giwa-Osagie F, Stevenson GW. Role of pelvimetry in active management of labour. Br Med J 1975;4:505-7.
3. Berkowitz RL, Fine EA. Is pelvimetry worthwhile? In: Symonds EM, Zuspan FP, eds. Clinical and diagnostic procedures in obstetrics and gynecology. Part A: Obstetrics. New York: Marcel Dekker, 1983:191-211.
4. Zatuchni GI, Andros GJ. Prognostic index for vaginal delivery in breech presentation at term. Am J Obstet Gynecol 1965;93:237-42.
5. Zatuchni GI, Andros GJ. Prognostic index for vaginal delivery in breech presentation at term. Prospective study. Am J Obstet Gynecol 1967;98:854-7.
6. Todd WD, Steer CM. Term breech: review of 1006 term breech deliveries. Obstet Gynecol 1963;22:583-95.
7. Beischer NA. Pelvic contraction in breech presentation. J Obstet Gynaecol Br Commonw 1966;73:421-7.
8. Rovinsky JJ, Miller JA, Kaplan S. Management of breech presentation at term. Am J Obstet Gynecol 1973; 115:497-513.
9. O'Leary JA. Vaginal delivery of term breech. Obstet Gynecol 1979;53:341-3.
10. Collea JV, Chein C, Quilligan EJ. The randomized management of term frank breech presentation: a study of 208 cases. Am J Obstet Gynecol 1980;137:235-42.
11. Flanagan TA, Mulchahey KM, Korenbrot CC, Green JR, Laros RK. Management of term breech presentation. Am J Obstet Gynecol 1987;156:1492-9.
12. Boice JD, Land CE. Ionizing radiation. In: Schottenfeld D, Fraumeni JF, eds. Cancer epidemiology and prevention. Philadelphia: WB Saunders, 1982:137-238.
13. Gimovsky ML, Willard K, Neglio M, Howard T, Zerne S. X-ray pelvimetry in a breech protocol: a comparison of digital radiography and conventional methods. Am J Obstet Gynecol 1985;153:887-8.
14. Kopelman JN, Duff P, Karl RT, Schipul AH, Read JA. Computed tomographic pelvimetry in the evaluation of breech presentation. Obstet Gynecol 1986;68:455-8.
15. Stark DD, McCarthy SM, Filly RA, Parer JT, Hricak H, Callen PW. Pelvimetry by magnetic resonance imaging. AJR 1984;144:947-50.
16. Dudenhausen JW, Pfammater T, Marincek B, von Schulthess GK, Huch A. Pelvimetry using magnetic resonance imaging. Geburtshilfe Frauenheilkd 1989;49:477-80.
17. Diehl J, Holmberg NG. The assimilation pelvis—a radiological and obstetrical study. Acta Obstet Gynecol Scand 1968;47(suppl 7):1-93.
18. Beers GJ. Biological effects of weak electromagnetic fields from 0 Hz to 200 MHz: a survey of the literature with special emphasis on possible magnetic resonance effects. Magn Reson Imaging 1989;7:309-31.


30 years ago,
Searle
urned women's heads
toward a new
method of birth
control.
WHAT IS
SEARLE DOING
TODAY?



MAKING A GREAT CASE FOR DEMULEN® 1/35



THE CASE FOR PRESCRIBING DEMULEN 1/35

- Low-dose formulation
- Minimal lipid impact
- Low incidence of some common androgenic side effects, such as acne, oily skin, and hirsutism
- Unique progestogen: ethynodiol diacetate
- No generic substitute available
- Covered by the Patient Promise® program

  If you prescribe Demulen 1/35, or any other Searle product, and do not achieve the desired therapeutic benefits, at any time, Searle will refund 100% of your patient's out-of-pocket cost for her most recent prescription for that product, *a full refund directly to your patient.*

DEMULEN® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Also available in the new Compack®
DEMULEN® 1/50 (ethynodiol diacetate 1 mg, ethinyl estradiol 50 mcg)

Please see adjoining page for a brief summary of prescribing information.

The physician should become familiar with the prescribing information (labeling) for this product, which discusses reported risks, such as thromboembolic disorders (including strokes and heart attacks), risk of estrogen dose, and hepatic lesions, as well as the need to monitor patients for early symptoms of any diseases or medical conditions, so use can be discontinued when appropriate. A brief summary of the prescribing information accompanies this advertisement.

SEARLE 30 YEARS EXPERIENCE IN ORAL CONTRACEPTION

© 1990, G.D. Searle & Co

A90DE3808T

DEMULEN® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

BRIEF SUMMARY

Demulen® 1/35: Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients. **Demulen® 1/50:** Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients.

Indication and usage: For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk*: Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen.

Contraindications: Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products.

Warnings:

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. Latter group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myocardial ischemia, occurs with high progestational activity. Consider amount of both steroids in choosing OC. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

Be alert to earliest manifestations of TE disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased er cancer with combination or progestogen-only OCs. Studies show no increased breast cancer with OCs or estrogens. greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found grea breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estro and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with factors and in various subgroups. Decrease in benign breast tumors in OC users is well known. Some studies increased risk of cervical dysplasia, CIN, erosion, and carcinoma in long-term users. Cervical microglandular hyper been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with O surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nor causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical **4.** *Hepatic adenomas, etc.* have been associated with OCs. In 1 study OC formulations with high "hormonal pote associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemop in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Lon users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdom tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur wit **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring:* Estrogens or progestogens d pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. 90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DE daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defe estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VA vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by ma was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Card defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,0 compared to 7.8 for those unexposed (P<.05). Female hormone exposure in utero was associated with testicular a cancer. More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence pro are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming preg post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon afte OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstr out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods a rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period an OCs. If pregnancy is confirmed, stop OC and apprise patient of potential fetal risks and discuss continuation of Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceiv this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregna **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 appeared in 6-12 months' use in another. Bile duct cancer has been reported. **7.** *Carbohydrate and lipid metabolism:* glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Trig total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in p familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated B in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for card disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of m development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluat **10.** *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discon BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent ble out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomer secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinui with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolo occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio to intrauterine pregnancies is no higher than in nonusers. **12.** *Breast feeding:* OCs may decrease quantity and quali A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until afte **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairmer to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Pr** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and p BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positi observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monito in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred afte troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver d and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; me anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise p of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests afte for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, una T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, tr phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolong on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mas climacteric. Cervical chlamydial and gonorrheal prevalence is increased; do not assume OC protects against chlam HIV seropositivity was associated with OC use. *Information for patients*. See patient labeling. *Drug interactions*. OC eff may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chlorar sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, a tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, aceta **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thromb and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrom adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities evidence of association between the following and OCs, though confirmatory studies have not been done: r thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug relate vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enl secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate toleranc candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or r premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, pa nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema mul nodosum, hemorrhagic eruption, herpes gestationis, hidradenitis suppurativa, hemolytic uremia, malignant hyp impaired renal function, acute renal failure, sometimes irreversible, itching, vaginitis, porphyria, PVCs, abnor pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus eryth rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant n endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been repo large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: prescribing information should be consulted prior to use and is available on request.**

5/14/90 • P9

SEARLE

G.D. Searle & Co.
Box 5110, Chicago, IL 60680

Address medical inquiries to:
G.D. Searle & Co.
Medical & Scientific Information Depart
4901 Searle Parkway
Skokie, IL 60077

Intraamniotic infection in the very early phase of the second trimester

Israel Goldstein, MD, Etan Z. Zimmer, MD, David Merzbach, PhD, Bezaleal A. Peretz, MD, and Etan Paldi, MD

Haifa, Israel

A total of 157 consecutive patients were studied in an effort to examine prospectively the incidence of asymptomatic intraamniotic infection in the early phase of the second trimester. All patients were referred for amniotic fluid karyotyping. In addition, the amniotic fluids were examined for Gram stain and were directly cultured on blood agar and MacConkey agar as well as in thioglycollate broth. We found positive amniotic fluid cultures in eight cases (5.09%); however, results of Gram stain examinations were negative in all amniotic fluid samples. The data indicate that there is no correlation between white blood cells in the amniotic fluid and positive amniotic fluid culture results. Only one pregnancy with positive amniotic fluid culture resulted in a septic abortion. Therefore we can suggest that intraamniotic infection can exist early in pregnancy, even with intact membranes, and in most cases without any clinical symptoms. (AM J OBSTET GYNECOL 1990;163:1261-3.)

Key words: Intraamniotic infection, microbiology, late abortion, amniocentesis, second-trimester infection

The notion that amniotic fluid remains sterile as long as the membranes are intact is no longer acceptable.[1-10] Recently many investigators have noted the presence of asymptomatic intraamniotic infection particularly associated with preterm labor in the second half of gestation.[2, 3] Bobitt and Ledger[4] were the first to report positive bacteriologic analysis of amniotic fluids obtained by amniocenteses from the amniotic cavity in women with intact membranes. The authors postulated the presence of unrecognized amnionitis, that is, positive amniotic fluid culture in an afebrile mother. Intraamniotic infection has been recognized as a major etiologic factor in preterm delivery.[4] The prevalence of positive amniotic fluid cultures has varied from 0% to 25.8%.[1-10] So far there has been no report of which we are aware on the incidence of asymptomatic intraamniotic infection in the early phase of the second trimester.

The study reported herein was conducted with a population of women with intact membranes; the women underwent amniocenteses for cytologic purposes at 15 to 19 weeks' gestation. The objectives of the study were: (1) to determine the microbiology of the amniotic fluid; (2) to establish the incidence of intraamniotic infection in this particular patient population; and (3) to ascertain pregnancy outcome.

From the Department of Obstetrics and Gynecology "B" and the Department of Microbiology, Rambam Medical Center, Technion Institute of Technology.
Presented at the Tenth Annual Meeting of the Society of Perinatal Obstetricians, Houston, Texas, January 23-27, 1990.
Received for publication February 23, 1990; revised June 14, 1990; accepted June 18, 1990.
Reprint requests: Israel Goldstein, MD, Department of Obstetrics and Gynecology "B," Rambam Medical Center, Haifa, Israel.
6/1/23209

Patients and methods

Patient population. A prospective study of the incidence of asymptomatic intraamniotic infection was conducted in a population of normal pregnant women referred for amniocentesis. Amniotic fluid was obtained by transabdominal amniocentesis from 157 patients with singleton pregnancies referred for karyotyping; gestational ages ranged from 15 to 19 weeks. The procedure was done in all cases under sonographic guidance by the same physician (I. G.). The indications for amniocentesis within the study population were: maternal age, 109 patients; low level of maternal serum α-fetoprotein, eight patients; high level of maternal serum α-fetoprotein, six patients; previous affected child, 18 patients; anxiety, eight patients; and miscellaneous, eight patients. The success rate for the amniocenteses was 100%, and in each patient no more than one puncture was necessary. Indications to perform amniocentesis were high level of maternal serum α-fetoprotein, low level of maternal serum α-fetoprotein, maternal age, previous affected child, and maternal anxiety.

Amniotic fluid processing for microscopic examination. Preparations for amniotic fluid puncture included mechanical scrubbing of patient's abdomen with 10% povidone-iodine and 70% ethyl alcohol for at least 4 minutes. Amniotic fluid was obtained under highly

Table I. Types of organisms isolated in the amniotic fluid

Indication	*Organism*	*Karyotype*	*Outcome*
Maternal anxiety	*Klebsiella* species*	46,XX	Term delivery
Previous affected child	*Klebsiella* species† *E. coli**	46,XY	Preterm delivery
Previous affected child	*E. coli*†	46,XY	Septic abortion
Previous Down syndrome	β-Hemolytic*	46,XX	Preterm delivery
Previous affected child	*Diphtheroides*‡	46,XY	Term delivery
High maternal serum α-fetoprotein	*Klebsiella* species*	46,XX	Preterm delivery
Maternal age	*Enterobacter* species§ *Acinetobacter anitratus*§	46,XY	Term delivery
Maternal age	*Acinetobacter anitratus**	46,XX	Term delivery

*Scant growth.
†Overabundant growth.
‡Intermediate growth.
§Abundant growth.

aseptic conditions in standard test tubes for direct Gram stain, direct cultures, white blood cell count, and karyotyping. The specimens were delivered in sterile tubes to the microbiology laboratory within 5 minutes and processed immediately; white blood cells were examined in the hematology laboratory. The tubes were without anaerobic environment. To facilitate semiquantitative evaluation, a loopful of the specimen was smeared onto a solid medium. Approximately 0.5 ml of amniotic fluid was added to thioglycollate broth (Difco, Detroit) for enrichment and anaerobic growth. Gram stain examination was performed with standard reagents (gentian violet, Lugol solution, alcohol-acetone solution, and fuchsin, Sigma, St. Louis). The direct cultures were smeared onto a blood agar base and MacConkey agar.

All media were incubated in an atmosphere containing 5% carbon dioxide at 35° C ± 2° C for 24 to 48 hours. The specimens were examined by trained microbiologists after 24 and 48 hours. Pure cultures at primary isolation were used for identification of the microorganisms. In case of mixed flora, these were again inoculated to isolate pure cultures. Identification of the organisms was made with accepted microbiologic methods.[12]

We used the following semiquantitative definitions for the presence of microorganisms in the amniotic fluid: scant growth, <10 colonies per plate; intermediate growth, 10 to 20 colonies per plate; abundant growth, 20 to 40 colonies per plate; and overabundant growth; >40 colonies per plate. The presence and absence of white blood cells were also noted.

Statistical analysis was made with 95% confidence interval for the frequency of positive cultures.

Results

Between November 1988 and October 1989, 157 healthy pregnant women underwent routine amniocentesis. The indications for amniocentesis within the study population were: maternal age, 109 patients; low maternal serum α-fetoprotein level, eight patients; high maternal serum α-fetoprotein level, six patients; previous affected child, 15 patients; cystic hygroma, two patients; anxiety, eight patients; miscellaneous, eight patients. Two of the pregnancies with negative amniotic fluid cultures resulted in late abortions. Eight patients (5.09%) had positive amniotic fluid cultures (95% confidence interval 1.7% to 8.5%).

One patient with a positive culture of *Escherichia coli*, underwent a septic abortion 2 weeks after amniocentesis. Cultures obtained from the intrauterine cavity and placenta during the process of abortion also tested positive for *E. coli*.

Microbiologic examinations provided the following results: five cases of scant growth, one case of intermediate growth, one case of abundant growth, and one case of overabundant growth (see Table I).

Comment

Although development of overt chorioamnionitis in women with intact membranes is rare, recent studies have shown a high incidence of positive amniotic fluid cultures in preterm labor with intact membranes.[1-3] The majority of the patients have not had clinical signs of

chorioamnionitis. Romero et al.[3] reported a 9.1% prevalence rate of positive amniotic fluid cultures in women with preterm labor and intact membranes. Previous studies with the use of amniocentesis to determine the microbiologic status of the amniotic cavity in patients with preterm labor and intact membranes have documented a mean positive culture rate of 13.6% (46/339), with a range from 0% to 25.8%.[7]

Although most patients in these studies were not responsive to therapy with tocolytic agents and were delivered of infants prematurely, in some cases labor was delayed for a significant interval of time. It is of interesting that there were no clinical signs of sepsis in the majority of the delivered infants.

Prevedourakis et al.[11] examined the amniotic fluid of 39 women with intact membranes and no sign of preterm labor at 30 to 39 weeks' gestation. In one case (2.56%) intraamniotic infection was detected; the organism was *Proteus*. The patient was delivered of an infant at term, and no pathologic signs were noted either in the mother or in the newborn infant during the postpartum period.

In the present study we did not examine the amniotic fluid for mycoplasma species. We found a 5.09% incidence of aerobic infection (95% confidence interval, 1.7% to 8.5%); no anaerobic bacteria were found.

In the most interesting case in our study the patient had amniotic fluid with a scant growth of *E. coli*. Despite the relatively small growth the fetus was aborted. The same bacteria were found later in cultures taken from the uterine cavity and the placenta.

Microorganisms in the amniotic fluid indicate the presence of either infection in the lower genital tract or invasion through maternal circulation. However, we do not know the origin of the microorganisms found in the amniotic fluid and we do not have an explanation for the relatively good outcome of the patients with positive amniotic fluid cultures.

The results of the study were unexpected, particularly in view of the fact that the patient population was asymptomatic and low risk. In each instance the amniocentesis procedure was performed by the same obstetrician under strict aseptic conditions. Furthermore, the microorganisms present in the positive amniotic fluid cultures are not found under usual circumstances on the abdominal wall. Therefore the possibility of external contamination appears to be negligible.

A possible explanation for the inability to detect the presence of microorganisms on culture media may be provided by the fact that antibiotics are routinely added to amniotic fluid culture media in the cytogenetic laboratory.

Further investigation should be undertaken. Whereas we can assume that antibiotic treatment may eradicate infection in the amniotic cavity, the clinician's dilemma remains—to treat or not to treat the patient with positive amniotic fluid culture.

In conclusion, we can suggest that intraamniotic infection can exist early in a pregnancy with intact membranes and in most cases without any clinical symptoms. We recommend bacteriologic evaluation of each amniocentesis; moreover, in cases with positive amniotic fluid cultures, antibiotic treatment might be indicated.

We gratefully acknowledge the assistance of Mrs. Judy Golub; without her cooperation this manuscript could not have been carried out.

REFERENCES

1. Miller JM, Pupkin MJ, Hill GB. Bacterial colonization of amniotic fluid from intact membranes. Am J Obstet Gynecol 1980;136:796-803.
2. Wahbeh CJ, Hill Gb, Eden RD, Gall SA. Intraamniotic bacterial colonization in premature labor. Am J Obstet Gynecol 1984;148:739-42.
3. Romero R, Sirtori M, Oyarzun E, et al. Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. Am J Obstet Gynecol 1989;161:817-24.
4. Bobitt JR, Ledger WJ. Amniotic fluid analysis: its role in maternal and neonatal infection. Obstet Gynecol 1978; 51:56-62.
5. Romero R, Kadar N, Hobbins JC, Duff GW. Infection and labor: the detection of endotoxin in amniotic fluid. Am J Obstet Gynecol 1978;157:815-9.
6. Gibbs RS, Blanco JD, Clair PJ, Castaneda YS. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J Infect Dis 1982;145: 1-8.
7. Bobitt JR, Hayslip CC, Damato JD. Amniotic fluid infection as determinated by transabdominal amniocentesis in patients with intact membranes in premature labor. Am J Obstet Gynecol 1981;140:947-52.
8. Gravett MG, Hummel D, Eschenbach DA, et al. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986;67: 2237.
9. Duff P, Kopelman JN. Subclinical intraamniotic infection in asymptomatic patients with refractory preterm labor. Obstet Gynecol 1987;69:756-9.
10. Guznick DS, Winn K. The association of chorioamnionitis with preterm delivery. Obstet Gynecol 1985;65:11-6.
11. Prevedourakis C, Papadimitrious G, Ioannidou A. Isolation of pathogenic bacteria in amniotic fluid during pregnancy and labor. Am J Obstet Gynecol 1970;106: 400-2.
12. Lennett EH, Balows A, Hausler WJ Jr, Shadomy HJ. Manual of clinical microbiology. 4th ed. Wasington DC: American Society for Microbiology, 1985.

Placental histology in fetuses between 18 and 23 weeks' gestation with abnormal karyotype

R. S. Kuhlmann, MD, PhD, A. L. Werner, MD, J. Abramowicz, MD, S. L. Warsof, MD, J. Arrington RDMS, and D. L. Levy, MD

Norfolk, Virginia

Placentas from karyotypically abnormal fetuses (18 to 23 weeks' gestation) were analyzed prospectively at the light microscopic level. Group I consisted of 14 control placentas. Group II consisted of 14 placentas from fetuses with an abnormal karyotype. Secondary and tertiary stem villi counts, small muscular artery counts, and total vessel counts were determined per 100× field. There were no differences in secondary and tertiary stem villi counts between groups. A significant decrease in small muscular artery counts ($p < 0.01$) and total vessel counts ($p < 0.01$) was noted in group II. Placental and fetal weights were comparable between groups. This undervascularization may represent placental immaturity as a result of arrested or delayed angiopoiesis. It appears that this abnormality is established before the third trimester and may be enhanced by late vascular obliteration as reported by others. These data substantiate the concept that the structure and function of the placenta is determined to a great degree by fetal karyotype and may help explain the morbidity and mortality seen in these fetuses. (Am J Obstet Gynecol 1990;163:1264-70.)

Key words: Placental undervascularization, abnormal karyotype

Fetuses with chromosomal abnormalities, with or without an associated structural defect, are often growth retarded and have an increased rate of intrauterine death.[1, 2] The cause of this increase in morbidity and mortality is not clear. It is believed that the growth disturbance is genetically determined in part by the fetus.[1, 3] Recent studies have quantitatively demonstrated a significant correlation between placental undervascularization and abnormal Doppler velocimetry in the trisomic[2] and growth-retarded but karyotypically normal fetus[4, 5] in the third trimester. Abnormal Doppler velocimetry has also been documented in fetuses with major structural anomalies, but placental correlation is lacking in these studies.[6, 7] Our pilot study revealed many of the karyotypically abnormal fetuses to have abnormal Doppler velocimetry between 18 and 23 weeks' gestation. We are not aware of any authors who have addressed placental histopathology at this gestational age in the karyotypically abnormal fetus.

The purpose of this study was to quantitatively evaluate the microvascular anatomy of the placenta to determine whether placenta undervascularization reported in the third trimester is present between 18 to 23 weeks' gestation in the karyotypically abnormal fetus. The presence of this abnormality may be responsible for abnormal blood flow in the umbilical artery during this period of gestation.

From the Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Eastern Virginia Medical School.
Received for publication April 6, 1990; revised June 19, 1990; accepted June 25, 1990.
Reprint requests: Randall S. Kuhlmann, MD, PhD, Department of Obstetrics and Gynecology, Medical College of Wisconsin, Milwaukee County Medical Complex, 8700 W. Wisconsin Ave., Milwaukee, WI 53226.
6/1/23411

Table I. Gestational age of groups I and II

Group	*No.*	*Weeks (mean ± SD)**	*Range (wk)*
I	14	21.3 ± 1.31	18-23
II	14	20.6 ± 0.84	19-22

*$p = 0.16$, NS.

Material and methods

Placentas were analyzed prospectively from two groups of fetuses from pregnancies terminated at 18 to 23 weeks' gestation. Group I consisted of 14 control placentas obtained from patients first seen at our tertiary care center in premature labor or with advanced cervical dilatation as a result of cervical incompetence. Group II consisted of 14 placentas from fetuses that were karyotypically abnormal with or without a structural defect. Placentas associated with abruptio placentae, clinical or pathologic evidence of chorioamnionitis, umbilical cords with one or two vessels, placenta previa, multiple gestations, hypertension, diabetes, and stillbirths were excluded from the study. Retained placentas were discarded unless 90% of the placenta was obtained intact by manual extraction. All pregnancies in

Table II. Abnormal karyotypes and associated structural defects found in group II

Abnormal karyotype	*No.*	*Structural defect*	*No.*
Trisomy 18	2	Encephalocele	1
Trisomy 21	4	Endocardial, cushion defect	1
Trisomy 22	1	Cystic hygroma	1
45,X	2	Cystic hygroma	1
47,XXY	1	None	0
69,XXX	1	None	0
46,XX,del(4)(p15.2)	1	None	0
46,XX,del(13)(q22)	1	Holoprosencephaly	1
46,XY,del(3)t(3;16)(p26,q23)	1	None	0

Table III. Comparison of placental and fetal weights of groups I and II

	*Placental weight (gm)**		*Fetal weight (gm)†*	
Group	*No.*	*Mean ± SD*	*No.*	*Mean ± SD*
I	8	204 ± 23	14	315 ± 61
II	9	193 ± 30	14	269 ± 70

*Placental weight I vs II, NS.
†Fetal weight I vs II, NS.

groups I and II had an ultrasonographic confirmation of gestational age. Placentas were weighed wet if intact and two or more tissue blocks were obtained 2 cm from the cord insertion and 2 cm from the placental edge after macroscopic evaluation by serial sectioning of the placenta at 1 cm intervals. Villous tissue that appeared unusual was avoided. Tissue blocks were fixed in 10% formalin, then embedded in paraffin. Sections (4 μm) were then stained with hematoxylin and eosin and trichrome. Slides stained with hematoxylin and eosin were used for initial evaluation and trichrome slides were used for counts because collagen and smooth muscle were better defined. Trichrome-stained slides were analyzed at magnifications of ×40 and then magnifications of ×100. Areas of infarct, fibrin deposits, and primary stem villi with anchoring stem villi were avoided. Midzone areas were analyzed and total vessel counts, small muscular artery counts, and secondary and tertiary stem villi counts[8] were established. Small muscular arteries (diameter up to 90 μm) and arterioles defined as vessels approximately capillary size (20 μm) with walls consisting of two layers of smooth muscle were counted per 100× field. Capillaries were also counted when secondary and tertiary stem villi counts were performed. Counts were reported per 100× field.

At least five fields per slide at a magnification of ×100 were counted by an anatomist-obstetrician (R. S. K.) and a pathologist (A. L. W.) who were not aware of gestational age and fetal status. Counting was repeated by each person on two occasions separated by at least a 1- to 2-week interval.

Doppler analysis. Ultrasound-guided continuous-wave Doppler analysis (Multigon 500A, Multigon Industries, Inc., Mt. Vernon, N.Y.) was performed on the umbilical artery in group II approximately 12 to 24 hours before pregnancy termination with prostaglandin vaginal suppositories. Absent end-diastolic flow or a resistance index ≥0.79 was considered abnormal on the basis of normograms used in our laboratory. Doppler analysis was not performed in group I because of advanced preterm labor.

Statistical analysis. The paired Student *t* test was used to evaluate the reproducibility of counting[4] and to establish possible intra- and interobserver differences. Fetal and placental weights were analyzed by a Student *t* test for independent samples. All counts in groups I and II were evaluated for a parametric or nonparametric distribution. Analysis of raw data revealed a nonparametric distribution and Mann-Whitney *U* analysis was used to determine whether differences existed between groups. In addition, modal counts were determined and analyzed by the Wilcoxon test as described in a previous study involving placental analysis in the third trimester.[4]

Results

Characteristics of groups I and II are depicted in Table I. Group II is further categorized with respect to karyotype and associated structural anomalies in Table II. Placental and fetal weights were comparable between groups I and II (Table III). Secondary and tertiary stem villi counts did not differ significantly between groups ($p = 0.19$). A significant decrease in small muscular artery counts ($p < 0.01$) and total vessel

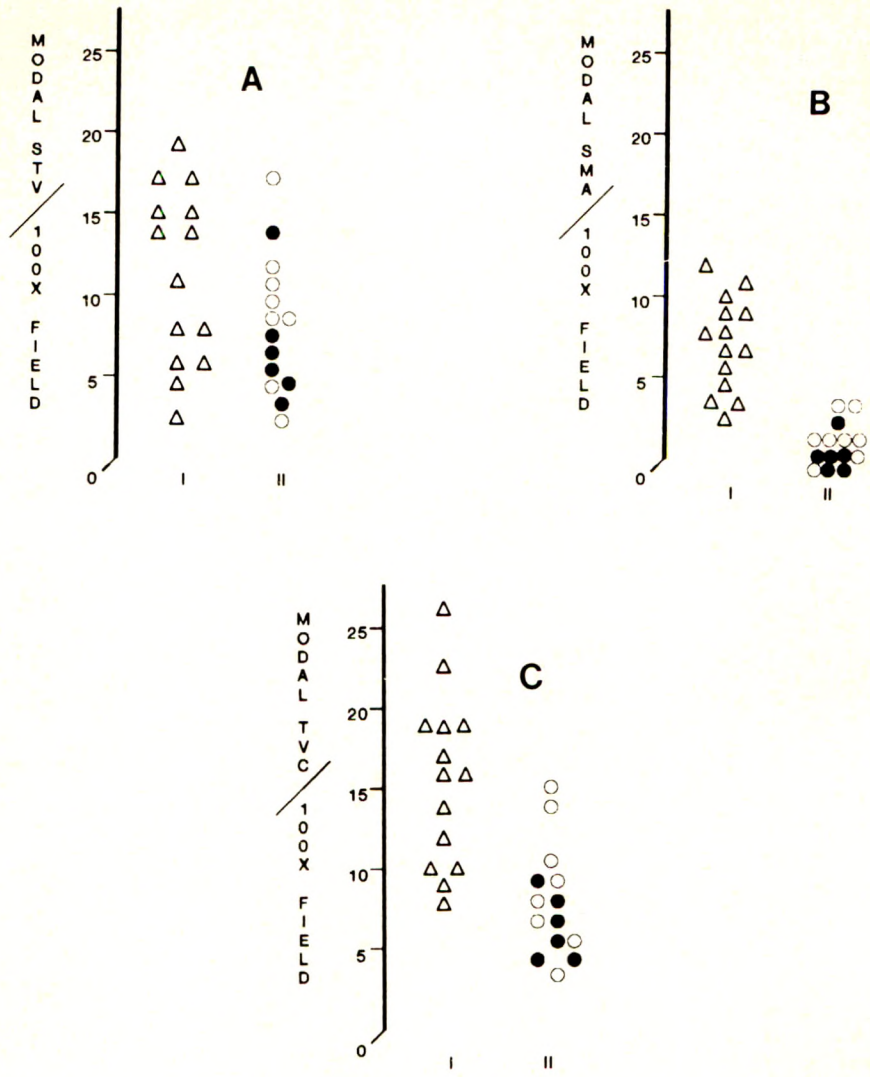


Fig. 1. A, Modal secondary and tertiary stem villi *(STV)*, **B**, small muscular artery *(SMA)*, and **C**, total vessel count *(TVC)*. *Triangles* represent group I; *circles* represent group II. *Solid circles* represent fetuses with abnormal Doppler velocimetry; *open circles* represent fetuses with normal Doppler velocimetry in group II.

Table IV. Comparison of secondary and tertiary stem villi counts, small muscular artery counts, and total vessel counts between groups I and II (mean ± SD)

Group	*No.*	*Secondary and tertiary stem villi counts**	*Small muscular artery counts†*	*Total vessel counts†*
I	14	9.3 ± 5.2	7.4 ± 2.9	15.7 ± 5.5
II	14	7.5 ± 3.5	3.1 ± 2.2	11.4 ± 4.9

*NS.
†$p < 0.01$ (Mann-Whitney U test).

counts ($p < 0.01$) was observed in group II as compared with group I (Table IV). No significant intraobserver differences existed between groups when counting was performed by both authors, (R. S. K.) and (A. L. W.), on two occasions separated by 1 to 2 weeks, demonstrating reproducibility of counting by each author. When interobserver comparisons were made, one author (R. S. K.) counted significantly more secondary

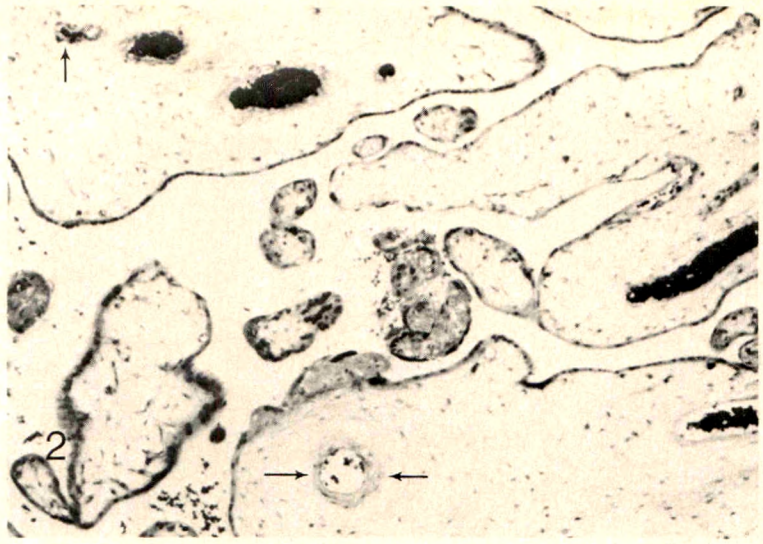


Fig. 2. Well-vascularized villi of control fetus at 22 weeks' gestation. *Double arrows* represent a muscular artery. *Single arrow* represents smaller muscular vessel and adjacent larger muscular vessel consisting of a smooth muscle bilayer. (Original magnification ×60.)

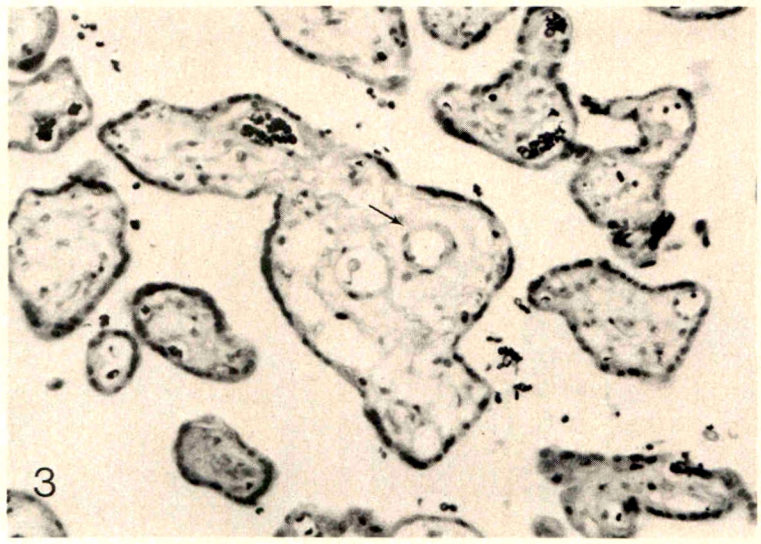


Fig. 3. Well-vascularized villi of control fetus at 23 weeks' gestation *Arrow* represents small muscular artery. (Original magnification ×60.)

and tertiary stem villi ($p = 0.047$) in both groups as compared with the other author (A. L. W.). There were no significant differences when small muscular artery counts and total vessel counts were compared. Because of the lack of intraobserver variation and minimal interobserver variation and the same final findings of each author in groups I and II, data from R. S. K. only was used in this study. Modal counts were plotted in Fig. 1. Modal secondary and tertiary stem villi counts did not differ significantly (Wilcoxon test, $p = 0.06$). A significant decrease in modal small muscular artery counts ($p < 0.05$) and modal total vessel counts ($p < 0.05$) did exist in group II as compared with group I when analyzed by the Wilcoxon test. Six of the 14 fetuses (43%) in group II were found to have abnormal Doppler velocimetry. Photomicrographs of representative fields are depicted in Fig. 2 to 7.

Comment

It has been postulated that the placenta is the fetal organ and its structure and function are determined to a variable degree by fetal karyotype in human beings[2]

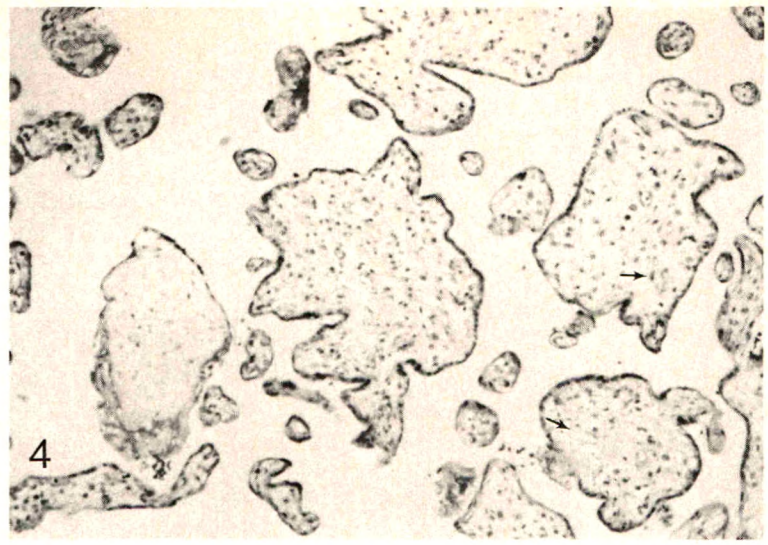


Fig. 4. Undervascularized villi of fetus with trisomy 18 at 22 weeks' gestation compared with Fig. 2. *Arrows* depict small vessels lacking walls composed of smooth muscle bilayer. (Original magnification ×60.)

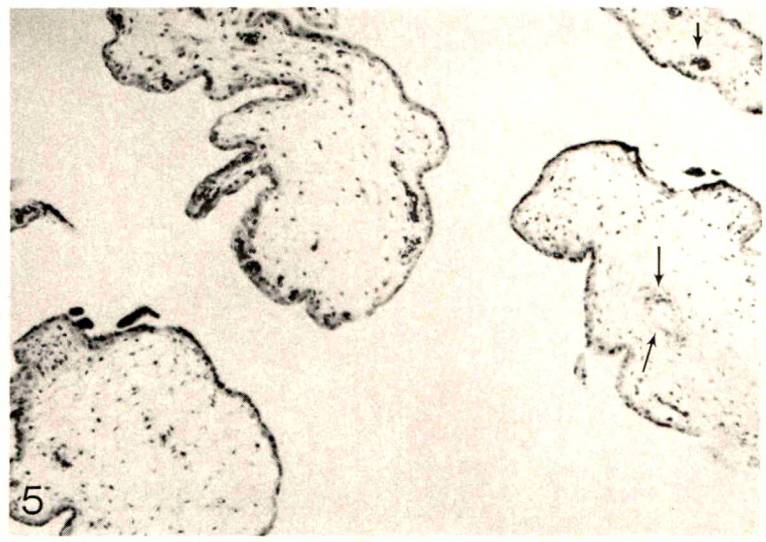


Fig. 5. Undervascularized villi of fetus with trisomy 21 at 22½ weeks' gestation compared with Figs. 2 and 3. *Double arrows* depict small muscular artery. *Single arrows* depict capillary. (Original magnification ×60.)

and certain animal models.[9] Abnormal growth seen in fetuses with aberrant karyotypes is believed to be determined in part by the genotype of the fetus.[1, 3] Structural abnormalities of the placenta may also contribute to abnormal fetal growth.[2, 4, 5] A significant decrease in placental small muscular arteries and the small muscular artery to villus ratio has been demonstrated in the fetus with autosomal trisomy and in the normal but growth-retarded fetus during the third trimester.[2, 4, 5]

In this study fetal weights were comparable between groups despite a trend toward decreased weight in group II. Whereas there were no significant differences among the groups regarding secondary and tertiary stem villi counts, the total vessel counts and small muscular artery counts were significantly less in group II. Modal secondary and tertiary stem villi counts did not differ significantly (Wilcoxon test, $p = 0.06$). This result may be influenced by the limited number of cases available. A further evaluation of a larger number of



Fig. 6. Villi of fetus with 45,X and cystic hygroma at 21½ weeks' gestation. Note undervascularization and increased villous edema compared with Fig. 2. *Arrow* depicts small blood vessel without smooth muscle bilayer. (Original magnification ×60.)

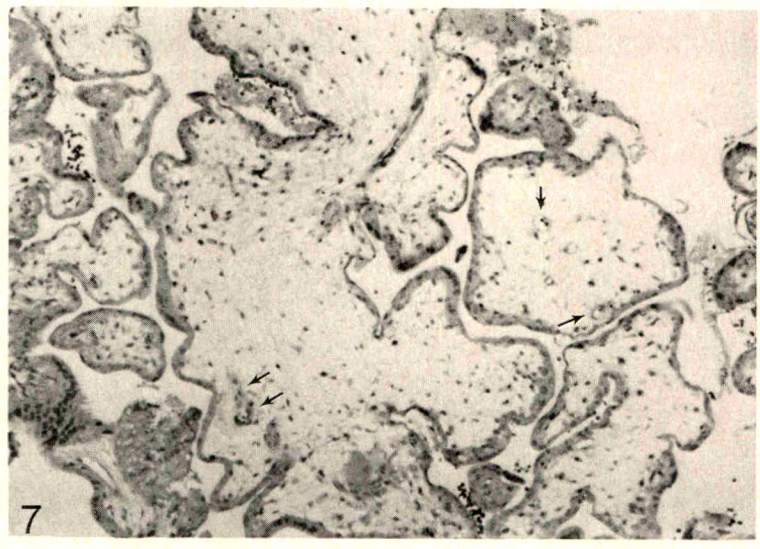


Fig. 7. Villi of fetus with trisomy 22 and cystic hygroma at 22 weeks' gestation. Villi appear to consist primarily of capillaries *(single arrows)*. *Double arrows* represent a small muscularized vessel. (Original magnification ×60.)

cases may demonstrate a significant difference. Previous authors[2, 4, 5] have also noted obliteration of vessels in addition to the actual decrease in numbers of small muscular arteries. Obliterated vessels were rarely seen in placentas of groups I and II. Many placentas in group II had few or no small muscular arteries present as previously noted in the third trimester.[2] Villous edema was noted in both groups. Qualitatively this edema appeared greater in placentas associated with trisomy and 45,X and particularly those associated with a cystic hygroma. This may represent immaturity and appears to decrease with advancing gestational age. The significance of the selective increase in edema seen in these specific cases is not clear.

Recent studies[2, 4, 5] have demonstrated that as the number of small muscular arteries decreases, placental vascular resistance increases with subsequent decreases in end-diastolic blood flow within the umbilical artery. This has also been reported in the karyotypically abnormal fetus in the third trimester.[2]

In our pilot study, many fetuses with congenital anomalies had abnormal Doppler velocity waveforms between 18 to 23 weeks' gestation. In this study 6/14 (43%) fetuses exhibited abnormal Doppler velocimetry. Doppler studies were not performed on fetuses in group I, therefore only inferences can be made. Although speculative, the undervascularization seen in this study may explain our previous and current Doppler findings. This undervascularization seen in group II may represent placental immaturity as a result of arrested or delayed angiopoiesis. It appears that this abnormal vascularization is established before and continues into the late phase of the third trimester. In addition, the abnormality is probably enhanced by arterial obliteration as demonstrated by the work of others.[2]

To our knowledge, these are the first data comparing these two homogenous, yet different, groups of fetuses between 18 to 23 weeks' gestation. These results, added to the work of others performed in the late phase of the third trimester,[2] further our understanding concerning the morbidity and potential mortality seen in the karyotypically abnormal fetus. Last, these findings further substantiate the concept that the placenta is a fetal organ and that its structure and function are determined to a significant degree by fetal karyotype.

REFERENCES

1. Lin CC, Evans MI. Intrauterine growth retardation. New York: McGraw-Hill, 1984.
2. Rochelson B, Kaplan C, Guzman E, Arato M, Hansen K, Trunca C. A quantitative analysis of placental vasculature in the third trimester fetus with autosomal trisomy. Obstet Gynecol 1990;75:59-63.
3. Jones OW. Genetic factors in the determination of fetal size. J Reprod Med 1978;21:305-13.
4. Giles WB, Trudinger B, Baird P. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlations. Br J Obstet Gynaecol 1985;92:31-8.
5. Bracero L, Beneck D, Kirshenbaum N, Peiffer M, Stalter P, Schulman H. Doppler velocimetry and placental disease. Am J Obstet Gynecol 1989;161:388-93.
6. Hata K, Hata T, Senoh D, Aoki S, Takamiza O, Kitas M. Umbilical artery blood flow velocity waveforms and associations with fetal abnormality. Gynecol Obstet Invest 1989;27:179-82.
7. Meizner I, Katz M, Lunenfeld E, Insler V. Umbilical and uterine flow velocity waveforms in pregnancies complicated by major fetal anomalies. Prenat Diagn 1987;7:491-6.
8. Lavery JP, ed. The human placenta: clinical perspectives. 1st ed. Rockville, Maryland: Aspen Pubs, 1987:25-45.
9. Bersu ET, Mossman H, Kornguth S. Altered placental morphology associated with murine trisomy 16 and murine trisomy 19. Teratology 1989;40:513-23.

Carbon dioxide laser energy disperses human papillomavirus deoxyribonucleic acid onto treatment fields

Alex Ferenczy, MD,[a] Christine Bergeron, MD, PhD,[b] and Ralph M. Richart, MD[b]
Montreal, Quebec, Canada, and New York, New York

The possibility of dispersing viral deoxyribonucleic acid during carbon dioxide laser treatment of human papillomavirus–containing genital infections has been investigated with a commercially available dot blot hybridization technique. The viral ribonucleic acid probes were specific for groups of human papillomavirus types 6/11, 16/18, and 31/33/35. Laser energy was delivered by continuous-wave mode and the plume of smoke was evacuated by a vacuum suction system. Samples were taken with Dacron swabs from lesional tissues of 43 patients as well as from the treated areas and from the 5 cm surrounding normal skin before and after laser vaporization. Human papillomavirus deoxyribonucleic acid was identified in swabs from 34 of 43 (79%) lesional tissues and 7 of 43 (16%) treatment fields. Although a trend for higher human papillomavirus deoxyribonucleic acid positivity in laser margins after therapy (7/43, 16%) than before (4/43, 9%) was observed, the rates were not statistically significant. It is concluded that carbon dioxide laser energy disperses human papillomavirus deoxyribonucleic acid onto treatment fields and the adjacent normal epithelium. Viral contamination of treated areas may be reduced by positioning the fume evacuator within 1 cm of the field of laser vaporization and cleaning the treated areas and surrounding tissue after therapy. (AM J OBSTET GYNECOL 1990;163:1271-4.)

Key words: Carbon dioxide laser, smoke, human papillomavirus DNA, hybridization

Among the many treatment modalities available for human papillomavirus (HPV)–containing genital lesions, carbon dioxide laser therapy became one of the most attractive techniques employed in current gynecologic practice.[1] Carbon dioxide laser energy is largely absorbed by intracellular water that is instantaneously turned to steam resulting in the vaporization of lased cells. However, intracellular proteins and nuclear deoxyribonucleic acid (DNA) are water-free and thus are carbonized rather than vaporized. The resulting plume of smoke contains charred and partially or totally thermocoagulated cellular debris.[2] The plume is evacuated from the treatment field by suction. However, depending on the evacuation system used and the mode of its application during laser vaporization procedures, variable amounts of plume content may be dispersed onto treatment fields. Previous analyses failed to indicate viability of the carbonized particulates including those derived from normal and malignant cells as well as those from verruca and bacteria.[2-4] Most recently, however, in vitro bioassays found infectious bovine papillomaviruses in the smoke from bovine warts treated either with the carbon dioxide laser or electrocautery.[5] Concern has been raised that carbon dioxide laser vaporization of genital warts may also be associated with HPV DNA contamination of both the patient and the operating personnel. HPV DNA was found by experiments of hybridization in the plume of smoke generated by the carbon dioxide laser energy that was used to vaporize genital HPV infections in both humans[6] and animals.[7]

The purpose of this study was to determine, with the aid of a dot blot hybridization technique, whether lasing genital HPV infections in the human results in viral contamination of treatment fields.

Material and methods

Forty-three patients, 38 women and 5 men, aged 18 to 56 (mean, 30) in whom the histologic study was consistent with HPV infections of the lower genital tract have been treated in the Laser Surgery Unit of the Reddy Memorial Hospital, Montreal, with a Merrimack 840 carbon dioxide laser (Sigmacon, Toronto). The lesional tissues were ordinary condyloma acuminatum in 26 patients and low-grade (flat condyloma/cervical intraepithelial neoplasia grade 1) and high-grade (cervical intraepithelial neoplasia grades 2 and 3) intraepithelial lesions in the remaining 17 patients. The le-

From the Departments of Pathology and Gynecology of The Sir Mortimer B. Davis Jewish General Hospital and The Reddy Memorial Hospital,[a] and Columbia University College of Physicians and Surgeons.[b]
C.B. is a former Fellow in Gynecologic Pathology, The Sir Mortimer B. Davis Jewish General Hospital.
Received for publication March 2, 1990; accepted June 1, 1990.
Reprint requests: Alex Ferenczy, MD, Department of Pathology, The Sir Mortimer B. Davis—Jewish General Hospital, 3755 Cote Ste. Catherine Road, Montreal, Quebec, Canada H3T 1E2.
6/1/22841

Table I. HPV DNA positivity of treatment fields and their margins before and after laser vaporization of anogenital lesions

	Patients	
	No.	*%*
Lesions	34/43	79
Laser crater	7/43	16
Laser margins		
Before therapy	4/43	9
After therapy	7/43	16*

*NS.

Table II. HPV DNA types by colposcopic appearance of anogenital lesions

	Lesions	
HPV type	*Flat**	*Acuminate*
6/11	1	21
16/18	5	1
30s	2	—
Mixed	2†	2‡
Negative	7	2
TOTAL	17	26

*Condyloma, intraepithelial neoplasia.
†Types 16/18, 30s.
‡Types 6/11, 16/18.

sional areas treated ranged from 2 to 18 cm^2 (mean, 7 cm^2). The margins of lesional tissues were defined by the use of 5% acetic acid solution and colposcopy. Laser vaporization was done in four different treatment sessions each including 10 or 11 patients. Thirty patients were treated while under general anesthesia and the remaining 13 patients had local anesthesia. Laser energy was delivered with continuous-pulse mode; power outputs ranged between 20 and 50 W and spot sizes from 2 to 4 mm with power densities ranging between 500 and 1250 W/cm^2. Laser vaporization was generally extended between 5 and 10 mm beyond the margins of lesional tissue treated. Depth of penetration ranged from 5 mm (in the cervix) and 2 mm (hairy skin of the vulva) to 1 mm (vagina, nonhairy skin of vulva, and perianal skin) as measured by a laser ruler from the normal, adjacent surface epithelium. After each procedure the crater and surrounding epithelium were wiped with 5% saline solution. The operating room contained a wall-mounted exhaust system for evacuation of air. The laser-generated plume of smoke was evacuated with the Stackhouse Point One System laser smoke filtration system (El Segundo, Calif.) with vacuum suction settings between moderate and high (6 and 9) and the distal end of the vacuum tube placed approximately 2 cm from the area treated at a given time. The plume of smoke produced during vaporization of the cervix and vagina was evacuated via a metal cannula welded on the inner surface of the upper blade of commercially available, rough-surfaced specula (Euro-Med Inc., Alameda, Calif., and Simpson-Basye, Wilmington, Del.).

Immediately before treatment, in all patients cellular samples for dot blot hybridization (ViraPap/ViraType, LTI, Gaithersburg, Md.) were obtained with saline solution–moistened Dacron swabs that were rubbed firmly against the surfaces of the lesional tissues. Samples also were taken from normal epithelium adjacent and circumferential to lesional tissues to a distance of 5 cm. Cellular sampling for HPV detection and typing was repeated after the end of each laser treatment session from the area treated (laser crater) and the normal laser margin after wiping both areas with 5% acetic acid.

The filter hybridization was performed with reagents and methods contained in the ViraPap human papillomavirus DNA detection kit according to the manufacturer's directions.

The accuracy of these tests for detecting HPV DNA compared with the standard reference method of Southern blotting has been assessed in a separate study. Specimens from 830 patients were collected and aliquots were analyzed by ViraPap and Southern blotting. The ViraPap test had a sensitivity of 94.5% and a specificity of 95.5% (S. Challberg, LTI, personal communication). In a similar analysis of specimens from 262 patients the ViraType test had a sensitivity of 97.4% and a specificity of 95.7% compared with Southern blotting (S. Challberg, personal communication). The limits of detection of these tests are approximately 3, 1, and 3 pg for ViraPap, ViraType, and Southern blotting, respectively. For statistical analysis we used the χ^2 contingency test. A p value of 0.05 was considered significant.

Results

Thirty-four of the 43 (79%) cases tested were positive for HPV DNA (Table I). There were more acuminate (26/43) than flat (17/43) genital HPV infections. Human papillomavirus DNA positivity was higher in acuminate (24/26, 92%) than in flat (10/17, 60%) HPV infections (Table II), as well as higher in external anogenital (27/31, 80%) than in internal cervical-vaginal (7/12, 60%) lesions (Table III). There were nearly as many HPV type 6/11–positive lesions (22/34, 65%) as type 16/18– and type 30s–positive lesions (12/34, 35%) (Table II). Both the treatment fields and the surrounding 5 cm laser margins after vaporization yielded similar HPV DNA positivity (16%) (Table I). The rate of HPV DNA positivity in laser margins was lower before laser vaporization (9%) than after therapy (16%) (Table I); however, it was not statistically significant.

Table III. HPV DNA types by anatomic distribution of anogenital lesions

	Lesion			
HPV type	*Cervix*	*Vagina*	*Vulva-anus*	*Penis*
6/11	1	—	11	1
16/18	3	—	3	—
30s	1	—	1	—
Mixed	1*	1*	1†	1†
Negative	5	—	4	—
TOTAL	11	1	20	2

*Types 16/18, 30s.
†Types 6/11, 16/18.

Comment

The results of this study indicate that carbon dioxide laser-generated energy disperses viral DNA during vaporization of genital HPV infections. This was evidenced by the presence of HPV DNA in the laser treatment fields. Although the rate of HPV DNA positivity in the surrounding 5 cm of normal epithelium before and after therapy was not statistically significant, nevertheless it was nearly twice as high after (16%) as before (9%) therapy. The results confirm and extend earlier observations on papillomavirus infections in cattle and humans.[5-7] In these experiments viral DNA was found in the plume of smoke generated by carbon dioxide laser energy during vaporization procedures in four of four (100%) bovine fibropapillomas,[7] two of seven (28.5%)[7] and five of eight (62.5%)[5] human plantar warts, and in one of five (20%) precanister filters used during laser therapy of 65 patients with anogenital HPV infections.[6] Similarly, in an earlier study seven of 20 patients (35%) had positive test results for HPV DNA in normal skin within 1.5 cm of lased anogenital condylomas or intraepithelial neoplasia.[8] The somewhat lower rate of HPV positivity in our current study and our prior study on the plume of laser smoke[6] compared with that reported by Garden et al.[7] and Sawchuk et al.[5] may largely be attributed to the different viral content of lesional tissue treated. Verruca vulgaris and bovine warts contain significantly more HPV DNA than genital warts,[9] thus providing for potentially higher rates of viral detection and contamination of treatment fields, respectively.

In our previous report[8] we interpreted our finding of HPV DNA in the laser margins as latent infection of normal skin. However, results of this study suggest that the detection of HPV DNA in normal epithelium adjacent to lesional tissue before and after laser vaporization may also be caused by or be a reflection of viral shedding and carbon dioxide laser energy–generated viral dispersion.

The biologic significance of HPV DNA in and near laser-treated fields is not clear because it is not known whether the HPV DNA detected in clinical studies is in the form of virions. Mature virions are required for the transmission and development of subsequent infections, such as genital condylomas and squamous neoplasia, and HPV DNA alone is not thought to be infectious. Unfortunately, at present there are no in vitro infectivity bioassays available for HPV. However, a sensitive in vitro bioassay for bovine papillomavirus is available.[10] With this system Sawchuk et al.[5] were able to determine that infectious bovine papillomavirus particles could be recovered from laser smoke as evidenced by their ability to induce morphologic transformation of mouse C-127 cells. In addition, in a previous HPV DNA tracing study,[8] we reported a statistically significantly ($p < 0.01$) increased likelihood for early recurrent disease (3 to 6 weeks after treatment) at or near laser margins with HPV DNA–positive normal skin adjacent to lased tissue compared with HPV DNA–negative laser margins. In this study we postulated that acute recurrences were a reflection of later expression of "latent HPV" in laser margins during periods of postlaser repair. The latter usually is completed by the sixth postlaser week. In this study we did not verify the results of our previous study.

If it is assumed that carbon dioxide laser energy dispersed HPV DNA may be responsible for acute recurrences, efforts should be made to reduce viral contamination of treatment fields as much as possible. The most cost-effective means of prevention of viral dispersion is to position the suction tube of the smoke evacuator to within ≤1 cm of the area being lased. At that distance over 98% of nonvaporized material is evacuated from the lased area.[11] The remainder of the plume of smoke and its contents may be trapped by surgical masks (Tecnol Lazer mask, Sweet Medical, Lafayette, Calif.) specifically designed for laser surgical procedures.[5]

It is possible that viral contamination may further be reduced by cleaning the treatment fields with viricidal solutions after laser procedures.

It is possible, although not proved, that the mode of

laser energy used may also influence the rates of viral dispersion. However, neither the study by Garden et al.[7] nor our previous study on precanister filters[6] were able to establish a relationship between the mode of carbon dioxide laser energy used and the rates of viral contamination of treatment fields. In both studies intermittent to continuous-pulse mode with focused and defocused beams were used with power densities ranging between 500 and 5000 W/cm.[2] Regardless of the lasing modality used, HPV DNA was present in treatment fields or the plume of smoke.

We did not study the super-pulse mode with very short exposure times. It may be useful to conduct experiments with different modes of laser energy delivery systems including super-pulse, and with the use of viricidal cleaning solutions to determine if either or both will diminish HPV DNA contamination rates of treatment fields and, perhaps, lower recurrence rates and better treatment results.

REFERENCES

1. Ferenczy A. Laser treatment of patients wth condylomata and squamous carcinoma precursors of the lower female genital tract. CA 1987;37:334-7.
2. Oosterhuis JW, Verschueren CJ, Eibergen R, Oldhoff J. The viability of cells in waste products of CO_2 laser evaporation of Cloudman mouse melanomas. Cancer 1982; 49:61-7.
3. Mihashi S, Jako GJ, Incze J, et al. Laser surgery in otolaryngology: interaction of CO_2 laser and soft tissue. Ann N Y Acad Sci 1975;267:263-94.
4. Voorhies RM, Lavyne MH, Strait TA, Shapiro WR. Does the CO_2 laser spread viable brain-tumor cells outside the surgical field? J Neurosurg 1984;60:819-20.
5. Sawchuk WS, Weber PJ, Lowy DR, Dzubow LM. Infectious papillomavirus in the vapor of warts treated with carbon dioxide laser or electrocoagulation: detection and protection. J Am Acad Dermatol 1989;21:41-9.
6. Ferenczy A, Bergeron C, Richart RM. Human papillomavirus DNA in CO_2 laser-generated plume of smoke and its consequences to the surgeon. Obstet Gynecol 1990; 75:114-8.
7. Garden JM, O'Banion MK, Shelnitz LS, et al. Papillomavirus in the vapor of carbon dioxide laser-treated verrucae. JAMA 1988;259:1199-1202.
8. Ferenczy A, Mitao M, Nagai N, Silverstein SJ, Crum CP. Latent papillomavirus and recurring genital warts. N Engl J Med 1985;313:784-8.
9. Grussendorf-Conen EI, Gissmann L, Holters J. Correlation between content of viral DNA and evidence of mature virus particles in HPV-1, HPV-4 and HPV-6 induced virus acanthomata. J Invest Dermatol 1983;81:511-3.
10. Dvoretzky I, Shober R, Chattopadhyay SK, Lowy DR. A quantitative in vitro focus assay for bovine papillomavirus. Virology 1980;103:369-75.
11. Trevor M. Presence of virus in CO_2 laser plumes raises infection concern. Hosp Infect Control 1987;6:166.

Emergency treatment with recombinant tissue plasminogen activator of pulmonary embolism in a pregnant woman with antithrombin III deficiency

F. Baudo, MD,[a] T. M. Caimi,[a] R. Redaelli, PhD,[a] A. M. Nosari, MD,[a] M. Mauri, MD,[b] G. Leonardi, MD,[b] and F. deCataldo, MD[a]

Milan, Italy

Thromboembolic complications during pregnancy are frequent in patients with congenital antithrombin III deficiency. We report on a 29-year-old patient with congenital antithrombin III deficiency and severe pulmonary embolism treated with recombinant tissue plasminogen activator. The diagnosis of antithrombin deficiency is retrospective. This case indicates that the risk of thrombolytic therapy in this clinical setting might have been overemphasized. (Am J Obstet Gynecol 1990;163:1274-5.)

Key words: Pulmonary embolism in pregnancy, recombinant tissue plasminogen activator therapy

From the Departments of Haematology[a] and Intensive Coronary Unit DeGasperis,[b] Ospedale Niguarda Cà Granda.
Received for publication January 17, 1990; accepted March 27, 1990.
Reprint requests: Dr. F. Baudo, Department of Haematology, Ospedale Niguarda Cà Granda, Milano, Italy.
6/1/21110

Thromboembolic complications during pregnancy are frequent in patients with congenital antithrombin III deficiency: 70% according to Hellgren et al.[1] Data on thrombolytic therapy for pulmonary embolism in pregnancy are scant and equivocal. It is generally accepted that the use of thrombolytic agents carries a high

risk of placental bleeding and fetal death and that their use should be restricted to cases of massive pulmonary embolism not amenable to surgical treatment. The article by Declos et al.[2] is at variance with this view. They treated a 19-year-old pregnant woman with pulmonary embolism with urokinase and obtained good therapeutic response without significant side effects. We report on a patient with congenital antithrombin III deficiency and severe pulmonary embolism treated with recombinant tissue plasminogen activator (Boehringer Ingelheim, Ridgefield, Conn.). The diagnosis of antithrombin III deficiency is retrospective.

The patient, a 29-year-old woman in week 35 of pregnancy, was admitted to the Intensive Care Unit because of dyspnea and tachycardia of acute onset. Arterial gas analysis showed P_{O_2}, 63.9 mm Hg; P_{CO_2}, 29.6 mm Hg; and arterial pulmonary pressure 60.0 mm Hg (n.v. 18 to 26 mm Hg). Pulmonary scintigraphy showed almost complete absence of perfusion of the right lung and hypoperfusion of the lower two thirds of the left lung. A diagnosis of pulmonary embolism was made.

Treatment was a 3-hour intravenous infusion of 100 mg of recombinant tissue plasminogen activator followed by 20,000 U/day of sodium heparin in a continuous intravenous infusion. Almost complete reperfusion of the involved areas was documented by pulmonary scintigraphy at the end of recombinant tissue plasminogen activator infusion. The following morning, 20 hours after the end of the recombinant tissue plasminogen activator infusion, the patient was delivered of a male infant by cesarean section. Surgery was uneventful. No placental bleeding occurred. At surgery the coagulation laboratory data were consistent with heparin therapy: activated partial thromboplastin time ratio, 1.90; thrombin time ratio, 1.77; reptilase time ratio, 1.03, and fibrinogen, 2.2 gm/L. The patient was discharged on day 15 on oral anticoagulant therapy (warfarin). The patient has a type I antithrombin III deficiency, concordant reduction of antithrombin III activity and antigen (0.53 U/ml) with a normal crossed immunoelectrophoresis pattern.

The newborn had the clinical picture of acute respiratory distress syndrome without bleeding signs. The hemostatic parameters were consistent with prematurity (thrombin time ratio, 1.05; prothrombin time ratio, 1.66; activated partial thromboplastin time ratio, 2.19; fibrinogen, 0.9 gm/L; prothrombin, 19%; proaccelerin, 14%; proconvertin, 17%; antihemophilic factor, 19%; plasma thromboplastin component, 14%; Stuart factor, 22%; antithrombin III, 16%; and fibrinogen degradation product 8 μg/ml). He died on day 14. Autopsy showed intracerebral, intracerebellar, and subarachnoid hemorrhages commonly found in premature infants who die of adult respiratory distress syndrome, unrelated to the thrombolytic therapy.

The patient's mother died at the age of 29 because of postpartum pulmonary embolism. The patient's father has a normal antithrombin III level.

The case reported by Declos et al.[2] and our own indicate that the risk of thrombolytic therapy in this clinical setting might have been overemphasized.

Pulmonary embolism is a major cause of maternal death even in women without hereditary deficiencies that predispose patients to thrombosis.

REFERENCES

1. Hellgren M, Tengborn L, Abildgaard U. Pregnancy in women with congenital antithrombin III deficiency: experience of treatment with heparin and antithrombin. Gynecol Obstet Invest 1982;14:127-41.
2. Declos GL, Davila F. Thrombolytic therapy for pulmonary embolism in pregnancy: a case report. AM J OBSTET GYNECOL 1986;155:375-6.

Collagen metabolism in gynecologic patients: Changes in the concentration of the aminoterminal propeptide of type III procollagen in serum

Ulla Puistola, MD, PhD,[a] Leila Risteli, MD, PhD,[b] Juha Risteli, MD, PhD,[c] and Antti Kauppila, MD[a]

Oulu, Finland

We have previously found the serum concentration of the aminoterminal propeptide of type III procollagen, an indicator of collagen metabolism, to be increased in advanced ovarian cancer. In this study we measured the serum aminoterminal propeptide of type III procollagen concentration in healthy women during the menstrual cycle and in patients with salpingo-oophoritis, leiomyomas, endometriosis, and benign ovarian tumors. The concentration was higher in the luteal phase than that in the follicular phase, suggesting an association of collagen metabolism with ovarian steroid hormones. Severe salpingo-oophoritis increased the serum level of the aminoterminal propeptide of type III procollagen with a decrease to normal during recovery. Elevated values were occasionally seen in endometriosis and leiomyomas. These findings indicate that the aminoterminal propeptide of type III procollagen is a relatively unspecific indicator of ovarian carcinoma. (Am J Obstet Gynecol 1990;163:1276-81.)

Key words: Menstrual cycle, endometriosis, ovarian cysts, salpingo-oophoritis, aminoterminal propeptide of type III procollagen

Type III procollagen is one of the most abundant constituents of interstitial connective tissue, and comprises about 20% of the collagenous framework of both the myometrium and the cervix.[1] It is synthesized in the form of a larger precursor protein, known as type III procollagen, which contains additional peptide extentions at both ends. These sequences are known as the aminoterminal and carboxyterminal propeptides of type III procollagen.

The aminoterminal propeptide of type III procollagen, measurable in serum by radioimmunoassay,[2, 3] can be set free from the procollagen molecules during collagen synthesis, but it can also remain attached to the rest of the molecule and thus be incorporated into collagen fibers. Thus the serum aminoterminal propeptide of type III procollagen antigens can partly be derived from the de novo synthesis of type III collagen and partly from the turnover of this collagen.[4, 5] The aminoterminal propeptide of type III procollagen test has been found useful in the follow-up of ovarian cancer patients in whom elevated aminoterminal propeptide of type III procollagen concentrations in serum are frequently found, which closely correlate with the clinical behavior of the disease.[6, 7] During pregnancy the aminoterminal propeptide of type III procollagen concentration in serum increases toward term, probably reflecting the increased connective tissue metabolism in the uterofetoplacental compartment.[8]

The serum concentration of the tumor marker CA 125 is increased in some nonmalignant gynecologic diseases (e.g., endometriosis and inflammatory diseases) as well as during pregnancy, and it varies somewhat according to the phase of the menstrual cycle.[9] To fully judge the usefulness of the aminoterminal propeptide of type III procollagen measurement as a tumor marker complementary to CA 125, we evaluated the expression of the aminoterminal propeptide of type III procollagen in several nonmalignant gynecologic diseases. This study reports our findings on patients with endometriosis, salpingo-oophoritis, leiomyomas, ovarian cysts, and cystadenomas. In addition, the variation in the aminoterminal propeptide of type III procollagen concentration during the normal menstrual cycle was assessed.

From the Department of Obstetrics and Gynecology[a] and the Collagen Research Unit, Departments of Medical Biochemistry[b] and Clinical Chemistry,[c] University of Oulu.
Supported in part by grants from the Medical Research Council of the Academy of Finland and from the Finnish Cancer Foundation.
Received for publication November 27, 1989; revised May 25, 1990; accepted June 15, 1990.
Reprint requests: Dr. Ulla Puistola, Department of Obstetrics and Gynecology, University of Oulu, Kajaanintie 52 A, Oulu, Finland SF-90220.
6/1/23238

Material and methods

Patients. Ten normally menstruating healthy women, ages 20 to 25 years, were followed up with repeated aminoterminal propeptide of type III procollagen measurements during one menstrual cycle. The serum samples were otherwise obtained every 2 days, but during the periovulatory time they were taken daily. Follicle maturation was estimated by ultrasonographic examination.

Twenty patients with salpingo-oophoritis were followed up in the hospital for 3 to 18 days, depending on the severity of the disease. Blood samples for the measurements of the aminoterminal propeptide of type III procollagen, C-reactive protein, and erythrocyte sedimentation rate were obtained every 2 or 3 days.

For preoperative serum aminoterminal propeptide of type III procollagen analysis, blood samples were obtained from 18 patients with uterine leiomyomas, 36 patients with ovarian cysts, 18 patients with ovarian cystadenomas, and 97 patients with endometriosis.

None of the subjects studied had any other disease including hepatic, rheumatic, or malignant diseases that could influence the serum aminoterminal propeptide of type III procollagen concentration.

All blood samples were obtained with the patients' permission and the research was carried out according to the provisions of the Declaration of Helsinki. The serum samples were stored frozen at −20° C until analyzed.

Radioimmunoassay for the aminoterminal propeptide of type III procollagen. The concentration of the aminoterminal propeptide of type III procollagen was measured in duplicate 200 μl serum samples with a recently described equilibrium radioimmunoassay.[3] The solid phase second antibody separation reagent was kindly provided by Farmos Diagnostica (SF-90460, Oulunsalo, Finland). The sensitivity of the test was 0.2 μg/L. Both the intraassay and the interassay variations were <5% at the concentrations found during the study. The reference interval (mean ± 2 SD) for healthy adults is 1.7 to 4.2 μg/L with no difference between men and women. The concentration is independent of age in adults.

Statistical analyses. A one-way analysis of variance was used to analyze the statistical significances of the differences between different groups. The significance was then tested with a modified Student t test with calculation of the critical t value by the Bonferroni method.

For comparison of the serum aminoterminal propeptide of type III procollagen concentrations in the different phases of the menstrual cycle we calculated the mean values for the follicular, periovulatory, and luteal phases (cycle days 1 to 10, 11 to 15, and 16 to 28, respectively) for each subject. The percentage of deviation of each individual value from the total individual mean was determined, and this percentage was the variable used in the statistical treatment of the results.

Results

In 10 healthy women who were followed up throughout one menstrual cycle the concentration of the aminoterminal propeptide of type III procollagen in serum varied significantly during the menstrual cycle (Fig. 1, *A* and *B*). The concentrations measured remained within the reference interval, but were somewhat lower in the follicular phase (days 1 to 10), increased toward ovulation (days 11 to 15), and elevated during the luteal phase (days 16 to 28). The difference between the follicular and the luteal phases was statistically significant ($p < 0.001$; Fig. 1, *B*).

Twelve of 20 patients who had salpingo-oophoritis had a severe disease with elevated erythrocyte sedimentation rate and C-reactive protein with accumulation of purulent fluid in the pelvic cavity. The serum concentration of the aminoterminal propeptide of type III procollagen exceeded the reference interval in eight patients; seven of them had a severe infection. After a slower increase than those seen in erythrocyte sedimentation rate or C-reactive protein, the serum aminoterminal propeptide of type III procollagen changes followed closely the behavior of these two indicators of the acute phase reaction (Fig. 2).

The 97 patients with endometriosis were grouped according to FIGO (International Federation of Gynecology and Obstetrics) staging of the disease (Fig. 3). The median concentrations of serum aminoterminal propeptide of type III procollagen were 3.7 μg/L in stage I (range, 2.1 to 7.6 μg/L; $n = 54$), 3.3 μg/L in stage II (range, 2.0 to 6.1 μg/L; $n = 24$), and 3.9 μg/L in stages III and IV combined (range, 2.0 to 6.2 μg/L; $n = 19$). The upper limit of the reference interval was exceeded by 13, three, and six patients, respectively, or in 23% of all. There were no significant differences between the groups, and none of the groups differed significantly from the reference population, which consisted of healthy Finnish blood donors.[3]

Of the 18 patients with uterine fibroids from whom serum samples were obtained before operation, six had elevated serum concentrations of the aminoterminal propeptide of type III procollagen (mean, 4.1 μg/L; range, 3.6 to 7.6 μg/L). The value did not correlate with the age of the patient or with the size of the fibroid.

In the group of 36 patients with benign ovarian cysts, there were no elevated aminoterminal propeptide of type III procollagen concentrations. The median value

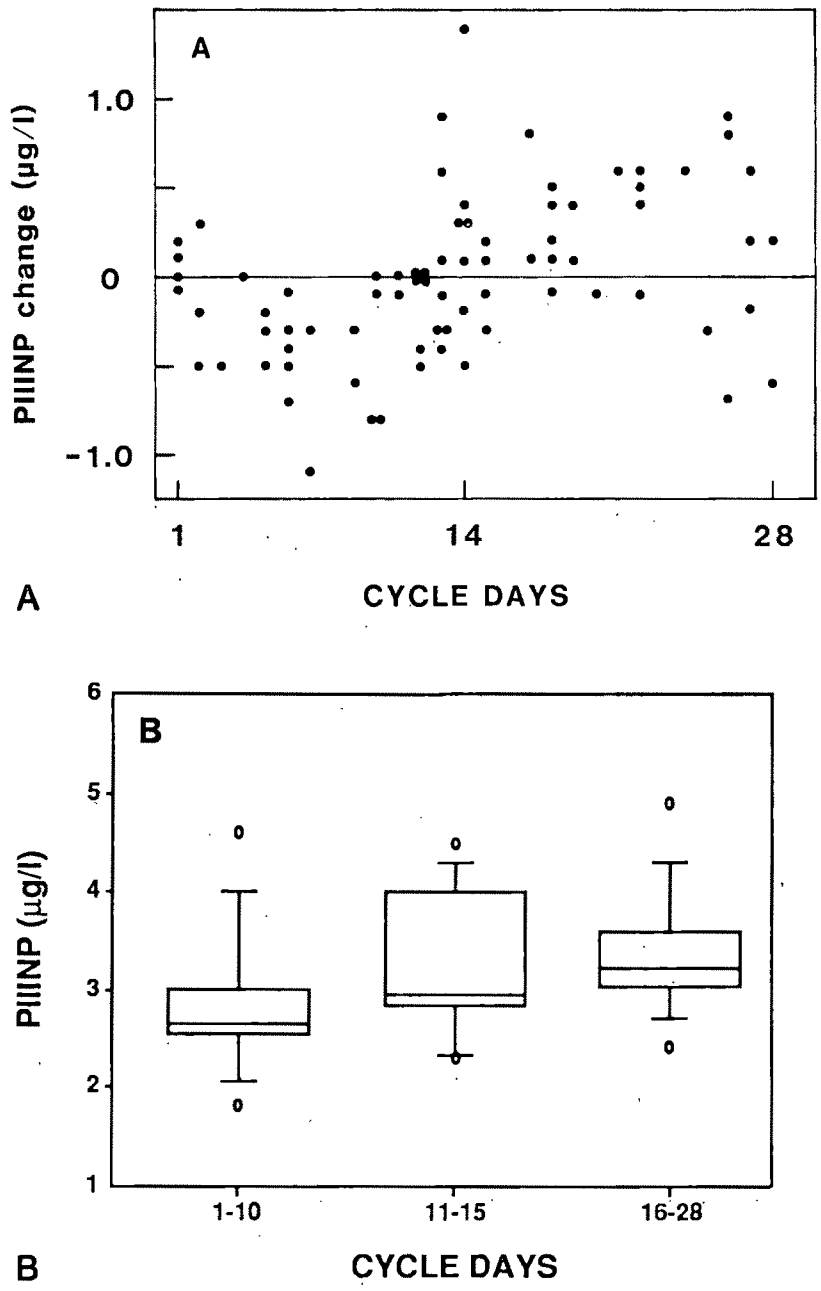


Fig. 1. Variation of aminoterminal propeptide of type III procollagen *(PIIINP)* concentration in serum during normal menstrual cycle. For detailed description of follow-up see Material and methods section. **A,** Deviation of individual values from overall mean value, calculated for each subject. **B,** Box and whiskers plot analysis. Central box covers middle 50% of values, whiskers through lower and upper quartiles extend to 10% and 90% percentiles. Highest and lowest individual values observed are indicated by *small circles. Horizontal lines* inside boxes are median values.

was 2.5 µg/L and the range was 1.4 to 4.1 µg/L. Of the 14 patients with a benign cystadenoma, only one had a aminoterminal propeptide of type III procollagen concentration above the reference range (5.8 µg/L). In this group the median was also 2.5 µg/L with a range of 1.6 to 5.8 µg/L.

Comment

The concentration of the aminoterminal propeptide of type III procollagen in serum is frequently increased in up to 83% of patients with advanced ovarian cancer and correlates with the clinical course of the disease.[6,7] In addition, it is elevated in about 40% of pa-

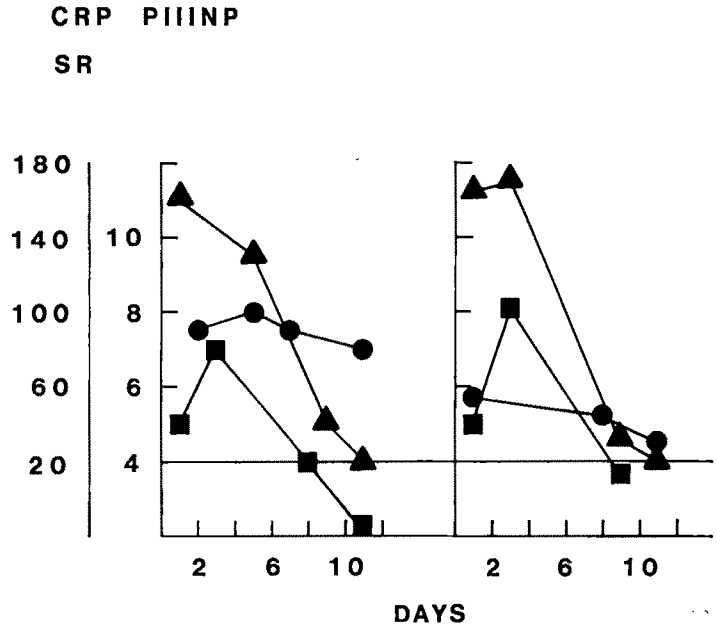


Fig. 2. Follow-up of two patients with severe salpingo-oophoritis. ▲ indicates C-reactive protein *(CRP)* (milligrams per liter), ● erythrocyte sedimentation rate *(SR)* (millimeters per hour) and ■ aminoterminal propeptide of type III procollagen *(PIIINP)* (micrograms per liter). *Horizontal line* indicates upper limits of reference intervals of these three parameters. Note different scales on left.

tients with advanced endometrial or cervical carcinoma.[10] The present study was undertaken to test whether the results of the aminoterminal propeptide of type III procollagen test are affected by other gynecologic diseases. This kind of information is essential for the correct clinical interpretation of elevated concentrations in patients with malignant tumors.

The nonmalignant disease that was most frequently associated with a significantly increased aminoterminal propeptide of type III procollagen concentration in serum was severe salpingo-oophoritis. In this septic condition the serum aminoterminal propeptide of type III procollagen concentration resembled the acute phase parameters of C-reactive protein and erythrocyte sedimentation rate in its behavior. A similar increase was found earlier in patients with septic wound infections.[11] In the latter case the serum antigen was characterized and found to be predominantly of larger size than the aminoterminal propeptide set free during synthesis of type III collagen. This finding suggests that the serum aminoterminal propeptide of type III procollagen in severe infections is derived from the partial degradation of type III collagen fibers in the affected tissues. Inasmuch as some aminoterminal propeptide is found on the surface of such fibers,[12] it is probably relatively sensitive toward locally increased proteolytic activity.

Like the CA 125 antigen, the aminoterminal propeptide of type III procollagen seems not to be affected by nonmalignant ovarian lesions. This is an important finding and it stresses the usefulness of the aminoterminal propeptide of type III procollagen test in the differential diagnosis between benign and malignant ovarian lesions.

A total of 23% of patients with endometriosis had serum aminoterminal propeptide of type III procollagen concentrations above the reference interval; however, there was no association between the elevations and the severity of the disease. CA 125 is also increased in about 25% of these patients, but in contrast to the aminoterminal propeptide of type III procollagen, the degree of elevation is related to severity of endometriosis.[9, 13] The reasons for the skewed distribution of the aminoterminal propeptide of type III procollagen values in our patients with endometriosis is not clear. It is possible that in this condition the aminoterminal propeptide of type III procollagen is derived from a reaction of peritoneal cells, including fibroblasts, to local irritation, a mechanism that could also function in salpingo-oophoritis. This may also be true for the increase in serum aminoterminal propeptide of type III procollagen in patients with uterine leiomyomas. Another source in this case might be the increased collagen metabolism inside the tumor.

Hormonal regulation of extracellular matrix metabolism is a clinically important but little investigated topic. The occurrence of the aminoterminal propeptide of type III procollagen antigen has been demonstrated

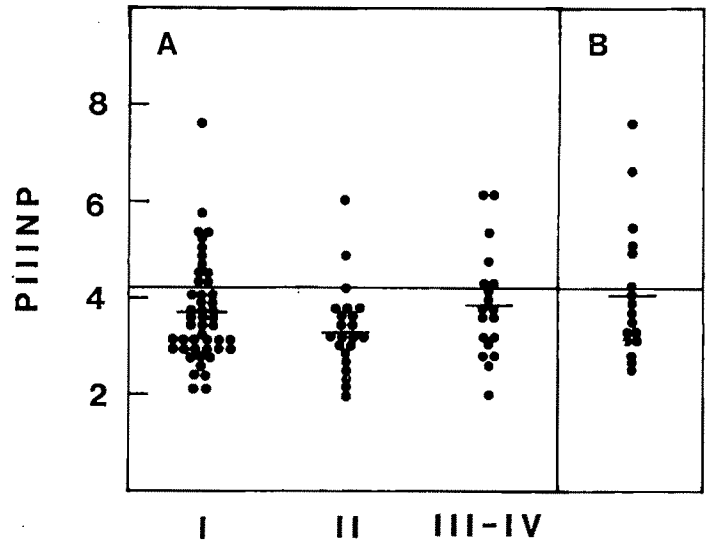


Fig. 3. Aminoterminal propeptide of type III procollagen *(PIIINP)* concentration (micrograms per liter) in serum of patients with endometriosis **(A)** and uterine leiomyomas **(B).** *I* to *IV* indicate stages of endometriosis according to FIGO (International Federation of Gynecology and Obstetrics) staging. *Continuous horizontal line* indicates upper limit of reference interval; *short horizontal lines* denote means for each group.

in ovarian follicular fluid, where its concentration correlates negatively with the progesterone content of the fluid.[14] We now found a statistically significant elevation of the aminoterminal propeptide of type III procollagen concentration in serum between the follicular and luteal phases of a normal menstrual cycle. This change, despite the concentration remaining all the time within the reference interval, was noted in each of the 10 women who underwent the study. Although the aminoterminal propeptide of type III procollagen values did not correlate with the estrogen or progesterone concentrations measured in the same blood samples, it seems obvious that the variation is a result of the hormonal changes and is brought about either by changes taking place in the endometrial connective tissue or by alterations in the matrix metabolism all over the body. It has been suggested that estrogens specifically repress the synthesis of type III procollagen in smooth muscle cells.[15] In addition, one of the principal functions of progesterone is thought to be an induction of the synthesis of stromal structures in the endometrium.

Our clinical studies revealed many similarities between CA 125 and aminoterminal propeptide of type III procollagen. Both are frequently increased in advanced ovarian carcinoma[6, 7, 9] and in some other gynecologic malignancies.[6, 9, 10] Increased concentrations of both markers are often present during pregnancy and pelvic inflammatory diseases and sometimes in endometriosis. The factors associated with the growth of gynecologic malignancies and with the inflammatory reactions of pelvic peritoneum seem to involve the regulation of the expression of both CA 125 and type III collagen.

We gratefully acknowledge the expert technical assistance of Kristiina Pekkala and Ulla Väinämö.

REFERENCES

1. Woessner JF. Uterus, cervix and ovary. In: JB Weiss, MIV Jayson, eds. Collagen in health and disease. Edinburgh, Scotland: Churchill Livingstone, 1982:506-27.
2. Rohde H, Vargas L, Hahn EG, Kalbfleisch H, Bruguera M, Timpl R. Radioimmunoassay for type III procollagen peptide and its application to human liver disease. Eur J Clin Invest 1979;9:451-9.
3. Risteli J, Niemi S, Trivedi P, Mäentausta O, Mowat AP, Risteli L. Rapid equilibrium radioimmunoassay for the aminoterminal propeptide of human type III procollagen. Clin Chem 1988;34:715-8.
4. Risteli L, Risteli J. Radioimmunoassays for monitoring connective tissue metabolism. Rheumatology 1986;10: 216-45.
5. Risteli L, Risteli J. Noninvasive methods for detecting organ fibrosis. In: M Rojkind, ed. Connective tissue in health and disease. Boca Raton, Florida: CRC Press, 1990:61-98.
6. Risteli L, Kauppila A, Mäkilä U-M, Risteli J. Aminoterminal propeptide of type-III procollagen in serum—an indicator of clinical behavior of advanced ovarian carcinoma? Int J Cancer 1988;41:409-14.
7. Kauppila A, Puistola U, Risteli J, Risteli L. Aminoterminal propeptide of type III procollagen: a new prognosis indicator in human ovarian cancer. Cancer Res 1989; 49:1885-9.
8. Risteli L, Puistola U, Hohtari H, Kauppila A, Risteli J. Collagen metabolism in normal and complicated pregnancy: changes in the aminoterminal propeptide of type

III procollagen in serum. Eur J Clin Invest 1987;17: 81-6.
9. Jacobs I, Bast RC. The CA 125 tumor-associated antigen: a review of the literature. Hum Reprod 1989;4:1-12.
10. Tomás C, Penttinen J, Risteli J, Risteli L, Vuori J, Kauppila A. Serum concentrations of CA 125 and aminoterminal propeptide of type III procollagen (PIIINP) in patients with endometrial carcinoma. Cancer 1990 [In press].
11. Haukipuro K, Risteli L, Kairaluoma MI, Risteli J. Aminoterminal propeptide of type III procollagen in serum during wound healing in human beings. Surgery 1990; 107:381-8.
12. Fleischmajer R, Perlish JS, Timpl R. Collagen fibrillogenesis in human skin. Ann NY Acad Sci 1985;460:246-57.
13. Kauppila A, Telimaa S, Rönnberg L, Vuori J. Placebo-controlled study on serum concentrations of CA 125 before and after treatment of endometriosis with danazol or high-dose medroxyprogesterone acetate alone or after surgery. Fertil Steril 1988;49:37-41.
14. Christiane Y, Lambotte R, Demoulin W, et al. Laminin and type III procollagen peptide in human preovulatory follicular fluid. Fertil Steril 1988;50:48-51.
15. Beldekas JC, Gerstenfeld L, Sonenschein GE, Franzblau C. Cell density and estradiol modulation of procollagen type III in cultured calf smooth muscle cells. J Biol Chem 1982;257:12252-6.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Why all placentas should be examined by a pathologist in 1990

Carolyn M. Salafia, MD,[a, b] and Anthony M. Vintzileos, MD[b]
Farmington, Connecticut

Placental pathology is rarely a part of the training for either obstetrician or pathologist. As a result there has been confusion regarding the potential benefits of routine placental examination. These benefits include clarification of the causes of many adverse pregnancy outcomes, improvement of the risk assessment for future pregnancies, and ascertainment of newborn risk for long-term neurodevelopment sequelae. Information on placental abnormalities may reveal the presence of chronic fetal insults and allow their differentiation from acute (peripartum) stresses. Current methods of risk assessment fail to identify the majority of pregnancies that end in prematurity, stillbirth, growth retardation, or fetal distress. We suggest that placental pathology should be a routine component of obstetric-neonatal care. (Am J Obstet Gynecol 1990;163:1282-93.)

Key words: Fetus, maternal-fetal medicine, placenta, pathology

It would surprise neither obstetricians nor pathologists if a governmental survey concluded that the single group expressing the most consistent interest in the placenta was the cosmetics industry, which purchases tons of placentas yearly for the extraction of hormones and proteins to be used in hair, face, and skin care treatments. There are several reasons for this mutual disinterest on the part of our medical colleagues. Placental and perinatal pathology is rarely a part of the residency training program for either discipline. The end result is lack of a mutual vocabulary, which leads to poor communication of relevant clinical information and frustration of both pathologist and obstetrician. The most significant problem with the pathologist is that he/she fails to appreciate the pathophysiologic features and clinical presentation of the histologic changes he/she may identify. As far as the obstetrician is concerned, he/she is most often unable to combine the clinical data with the different histologic types, which have to be merged in a clinically relevant fashion. In addition, obstetricians usually consider any information obtained from the study of the placenta to be retrospective, i.e., after the fact and therefore of no help in the prospective management of the pregnancy in question. Finally, adequate examination of the placenta requires an outlay in technical personnel and material, which, in an era in which cost-effectiveness is a major concern to all hospitals, may not be readily forthcoming.

It is our belief that examination of the placenta should be of vital interest to both obstetricians and neonatologists, as well as hospital lawyers and risk managers. The possible benefits derived from placental examination include: (1) clarification of the pathophysiologic features and significance of antepartum events detected by modern technology, as well as the relative contributions of "acute" (peripartum) and "chronic" (antepartum events of adverse neonatal outcomes; (2) improved management of subsequent pregnancies by diagnosing pathologic conditions that may have risks of recurrence or may even be preventable or treatable in subsequent pregnancies; (3) assessment of newborn risk for long-term neurodevelopmental sequelae, thus giving the opportunity to optimize long-term outcome by early and timely interventions.

This communication was therefore undertaken to address: (1) the terminology and clinical significance of gross and microscopic placental lesions, (2) the role of placental pathologic examination in differentiating between acute and chronic fetal insults, (3) the need for routine placental pathologic examinations in all preg-

From the Department of Pathology[a] and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[b] University of Connecticut Health Center.
Reprint requests: Carolyn M. Salafia, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, University of Connecticut Health Center, Farmington, CT 06032.
6/1/22481

nancies; and (4) the cost-effectiveness of routine placental pathologic examination.

Terminology and clinical significance of placental lesions

Gross examination (Table I)

Extraplacental membranes. The extraplacental membranes are examined for adherent blood clot, which may indicate the presence of abruptio placentae. The membranes' color may be described as "clear" (normal), cloudy, or opaque. When cloudy or opaque membranes are found, an ascending intraamniotic infection should be suspected. Membranes may also be described as green (indicating meconium staining), yellow (indicating amniotic hyperbilirubinemia) or red-brown (raising the possibility of intraamniotic bleeding). Certain bacterial infections may cause the membranes to appear green or yellow in the absence of intraamniotic pigments, but these membranes are generally also thick and opaque.

If the membranes are complete, the site of rupture should be determined by placing the hands inside the amniotic sac. The rupture site in a normal vaginal delivery should indicate the pericervical membranes. If the rupture site is at the placental margin, this suggests that the edge of the placenta may have been in the cervical area (a marginal placenta previa) or in the lower uterine segment. If the site of rupture in a vaginal delivery is >10 cm from the placental margin, it is likely that the implantation was fundal.

The membrane insertion onto the chorionic plate is described as marginal (normal), circummarginate, or circumvallate. When the membranes are inserted marginally, the chorionic plate extends to the edge of the placental disk, and the membranes are inserted at the edge of the disk. In circummarginate insertion the chorionic plate is circumscribed by a ring of fibrin. The placental disk may extend several centimeters beyond the limits of the chorionic plate. This insertion is not associated with fetal-neonatal compromise.[1] In circumvallate insertion the chorionic plate is also smaller than the placental disk, but the ring limiting the chorionic plate is a fold of extraplacental membranes (amnion, chorion, and decidua). The firm white appearance of this ring is due to ischemic necrosis of the chorion and decidua entrapped in this fold. Circumvallate insertion has been associated in our experience with chronic oligohydramnios, amnion rupture, maternal smoking, and other conditions of uterine vascular insufficiency.

Next, the amnion should be inspected carefully for the presence of amnion nodosum, a lesion seen in association with prolonged oligohydramnios of any cause. Grossly, these lesions appear as irregular lumps of granular material that cannot be easily scraped from the amnion surface.

The determination of chorionicity is very important in multiple gestations. For more information, the reader is referred to standard reviews of the subject.[1-3]

Table I. What to look for in gross placental examination

- Extraplacental membranes
  - Adherent blood clot
  - Color
  - Transparency
  - Site of rupture (relative to placental margin)
  - Insertion (marginal, circummarginate, circumvallate)
  - Amnion nodosum
  - Velamentous vessels (if present, ? intact)
  - Chorionicity (multiple gestations)
- Umbilical cord
  - Length
  - Diameter
  - Color
  - Vessel number (single artery)
  - Angioma, allantoic and omphalomesenteric remnants
  - True knots
  - Insertion (central, marginal, velamentous)
- Chorionic plate
  - Color and transparency
  - Dimensions and shape
  - Vascular pattern (velamentous vessels)
  - Subchorionic thrombi
- Maternal surface
  - Completeness
  - Adherent blood clot, placental indentation (abruptio placentae)
  - Calcifications
- Villous parenchyma
  - Infarction, intervillous thrombosis
    - Color
    - Location and dimensions
  - Chorioangioma
    - Color
    - Dimensions
    - Solitary-multiple
  - Maternal floor infarction
  - Perivillous fibrin deposition
  - Pallor

Umbilical cord

LENGTH. In term gestations the normal umbilical cord length ranges from 54 to 61 cm.[4-6] On the basis of Gardiner's calculation,[7] 32 cm is considered the minimal length of the cord in a term infant. Abnormally short cords have been observed in conditions of restricted fetal movements, such as in oligohydramnios, fetal paralysis, etc.[8] Long umbilical cords (arbitrarily set at >100 cm[6]) may be associated with multiple nuchal cords, cord prolapse,[9] or funic presentation.

DIAMETER. The umbilical cord diameter is usually larger where it is closer to the fetus and where it might be expected to have the greatest arterial pressure. Variations in umbilical cord diameter observed after the cessation of umbilical blood flow reflect to a large degree changes in the volume of Wharton's jelly. Both segmental lack of Wharton's jelly and lack of Wharton's jelly before insertion of the umbilical cord onto the chorionic plate ("furcate" or "forked" insertion) have

been seen in association with fetal compromise or death.[10] An increase in Wharton's jelly may appear as edema, with the umbilical cord taking on a milky color. Edema of the cord may be observed in cases of intraamniotic infection or a functionally significant true knot, as well as in cases of maternal diabetes and Rh isoimmunization.[11] In situations of fetal volume depletion, such as preeclampsia or intrauterine growth retardation, Wharton's jelly will be water depleted, and the surface of the umbilical cord will appear wrinkled (like "dishpan hands").

COLOR. The umbilical cord may also be stained by the same intraamniotic pigments (meconium, bilirubin, hemosiderin) that may stain the membranes. In cases of intrauterine fetal death, the umbilical cord will become progressively red-brown, as a result of hemolysis of fetal blood in the umbilical vessels. In cases of intraamniotic candidiasis, yellow plaques on the surface of the umbilical cord should alert the pathologist to the possibility of candidal abscesses.

UMBILICAL VESSELS. The normal umbilical cord contains two small caliber arteries and one larger caliber vein. Absence of an umbilical artery was originally reported to be significantly more frequent among infants with congenital anomalies, especially of the urinary tract, as well as with tracheoesophageal fistulas and central nervous system changes.[11] However, a single umbilical artery is also observed in clinically normal infants, and most frequently in our experience it is the only abnormality found. While Altshuler et al.[12] have shown no significant changes in incidences of fetal anomalies in cases of arterial aplasia versus arterial atrophy, we have observed several instances in which fusion of the umbilical arteries was confined to a single area of umbilical cord in otherwise normal infants. The relationship of a single umbilical artery to fetal anomalies may vary, depending on whether two functional umbilical arteries are present intraabdominally.[12]

OTHER UMBILICAL STRUCTURES. Other structures present in the umbilical cord may create the appearance of accessory vessels. Four or even five vessels may be apparent on cross section of the umbilical cord. While this may represent an area of varicosity, it may also be due to persistent omphalomesenteric vessels. A persistent allantoic duct may also appear to be a "fourth vessel." Umbilical angiomas are not infrequent microscopic findings but rarely are grossly recognizable or clinically relevant. Unusual lesions of the umbilical cord have been described, including teratomas.[1, 11, 13, 14]

TRUE KNOTS. True knots of the umbilical cord must be distinguished from "false knots" or varicosities, distinction generally made with ease. Cessation of blood flow through the umbilical cord by an acute tightening of a knot is a rare event if the blood flow through the umbilical cord has previously been normal. Tightening of a knot leading to restricted flow occurs only after the flow has already become abnormally slow or when the cord has already demonstrated decreased intravascular pressure. The mechanics and arrangement of the umbilical cord vessels are such that the cord acts as erectile tissue, which is not deformable under situations of high-volume blood flow.[15] After flow has ceased (i.e., stillbirth), a cord knot will often tighten during the labor and delivery, thus giving the false impression that obstruction to umbilical blood flow was the cause of fetal death. The clinical significance of a cord knot therefore must be carefully investigated. If a cord knot was primarily responsible for alterations in umbilical flow, several changes should be grossly apparent, depending on whether the changes were chronic or acute. If Wharton's jelly is tubular and not deformed, such a knot should be considered to be postmortem and therefore of dubious clinical significance. If the knot was tight in utero, umbilical venous distention should be present on the placental side of the knot, and edema, as a result of increased arterial pressure, should be present on the fetal side of the knot. If cord vessels are acutely obstructed by the knot while the fetal heart is actively beating, umbilical arterial hemorrhages on the fetal side of the knot and venous hemorrhage on the placental side of the knot might also be observed. In situations of chronic alteration of blood flow, both grossly and microscopically, thrombi may be observed as secondary events. Knots tightened postmortem should not have changes of edema, vascular distention, or disruption, and the proximal and distal segments of the cord should have a similar appearance.

UMBILICAL CORD INSERTION. The umbilical cord insertion may be described as central, eccentric, marginal, or velamentous relative to the chorionic plate. Eccentric cord insertions are associated with multiple gestations or uterine septa. Marginal and velamentous insertions may pose hazards to the fetus.[16] A marginally inserted cord may be more poorly supported than one based on the chorionic plate and more susceptible to compression from mechanical pressures. We have observed several cases of growth failure, as well as stillbirth, after external cephalic version when a marginal cord insertion was present. Placental abnormality was consistent with intermittent instability or changes in volume and pressure of flow within the placental circulation. The dangers of a velamentous cord insertion are well known. Velamentous vessels running in the placental membranes are also more susceptible to mechanical compression and, more important, to tearing during labor and delivery. The condition known as vasa previa, when velamentous vessels cross the cervical os, may be fatal to the fetus if not detected before membrane rupture.

Chorionic plate. The chorionic plate is composed of

amnion and the vascular chorion, within which there are arteries ramifying from the umbilical vessels and veins draining the placental villi. Inspection of the chorionic plate may reveal several characteristics of the extraplacental membranes, such as color and transparency. Abnormalities of insertion of the extraplacental membranes may result in abnormalities of the chorionic plate. A "battledore" placenta is one with an extremely eccentric cord and is of no significance unless velamentous vessels exist. White plaques may be visible through the chorionic plate. These are areas of subchorionic thrombi. They are more frequent in placentas of mothers who smoke.[17]

Maternal surface. Gross defects may indicate significant volumes of retained villous placenta. Clinically, the possibility of retained villous tissues may alert the obstetrician to patients at risk for postpartum hemorrhage or endometritis.

The maternal surface should also be examined for the presence of adherent blood clot. Any adherent blood must be considered as potentially relating to acute abruptio placentae, and when the clinical suspicion warrants, villous tissue adjacent to areas of blood clot should be examined for microscopic evidence of acute alterations in intervillous flow. The nearby maternal surface should be inspected for evidence of depression or compression of the villous placenta. A section through the villi subjacent to an area of adherent retroplacental blood should be examined microscopically for changes indicative of early infarction or focal trophoblast ischemia.

Villous parenchyma. The placental villi should be examined by serial sections of the placenta, perpendicular to the maternal surface and chorionic plate, at approximately 1 cm intervals. Both surfaces of each cut should be examined for the presence of the abnormalities described here.

INFARCTION. Recent (acute) infarctions may be difficult to distinguish from viable placenta but will generally be firmer and have a slightly different hue than adjacent viable areas. Old infarcts will be tan-white. All lesions suspected grossly to be infarctions should have this impression confirmed microscopically, since other lesions (for instance, infarcted chorioangiomas) may grossly resemble placental infarctions. The approximate size of the infarct should be noted. In a series of 500 consecutive placentas delivered of uncomplicated pregnancies at term, we observed a 10% incidence of placental infarction, and 90% of these lesions occupied <1 cm cubic volume.

The location of the lesion should be described as marginal (within 2 to 3 cm of the edge of the placenta) or central. In our experience 90% of placental infarctions in normal term deliveries are marginal. Whether a lesion is juxtabasal, subchorionic, or located centrally within the villous parenchyma is also important. The most likely location for ischemic villous death in the event of decidual vascular occlusion is at the maternal surface. Lesions grossly having the appearance of infarctions, which are located separate from the basal plate, should be carefully examined microscopically for the presence of associated acute or chronic villous inflammation.

INTERVILLOUS THROMBOSIS. In the subchorionic area blood flow is turbulent.[1] If spiral arterial flow is disturbed, subchorionic blood flow might become stagnant, possibly leading to subchorionic thrombosis. Subchorionic thromboses are common in term pregnancies and appear as laminated, white-tan plaques that can be seen through the chorionic plate. Generally, these lesions will be <5 to 8 mm in thickness and occupy less than 2 cm.[2] of the chorionic plate. Plaques of subchorionic fibrin have been observed more frequently among smokers,[17] who have increased numbers of platelets and increased platelet adhesiveness.[18] Intervillous thromboses that are distant from the maternal surface are usually composed of mixed maternal and fetal blood, and they are more common in cases of rhesus isoimmunization.[2] Intervillous thromboses close to the maternal surface are predominantly composed of maternal blood and may represent the effects of local disruption of spiral arterial flow.[19] While the clinical significance of intervillous thromboses has not been entirely understood, we have observed such thrombi, generally multiple and parabasal, in cases of preterm rupture of membranes with abnormal fetal biophysial assessment.

CHORIOANGIOMA (PLACENTAL HEMANGIOMA). These lesions may frequently be confused with intervillous thrombosis or placental infarction. Depending on the viability and vascularity of the lesion, they may appear as dark red-brown or white-yellow, sharply circumscribed lesions. Chorioangiomas may be solitary or multiple. If multiple, they may occasionally be associated with fetal angiomas in brain, liver, or other viscera.[20] They may also cause fetal compromise by their ability to function as shunts. Massive or multiple chorioangiomas may be found in cases of intrauterine death with or without fetal hydrops. Solitary lesions may present clinically as fetal tachycardia, as fetal distress, or, when infarcted, with mild degrees of neonatal thrombocytopenia, as a result of increased platelet consumption within the vascular tumor. Placental chorioangiomas have been assocated with extremely elevated maternal serum α-fetoprotein values and may be detected ultrasonographically.

MATERNAL FLOOR INFARCTION. This particular lesion is discussed separate from placental infarction because of its different etiology. Grossly, the lesion is generally a bandlike area of white-tan tissue running along the

Table II. What to look for in microscopic examination of placenta

Noninflammatory changes of amnion
Amnion nodosum
Meconium histiocytosis
Acute inflammatory changes
Pattern of spread of organism (extraamniotic or intraamniotic)
Maternal versus fetal inflammatory response
Discordance between maternal-fetal inflammatory responses
Infarction, intervillous thrombosis
Age
Associated pathologic condition (chronic villitis, decidual thrombosis, etc.)
Chronic villitis
Type of cellular infiltrate
Presence of intervillositis
Volume of affected tissue (grade)
Associated features (vasculitis, intravascular thrombi, hemorrhagic endovasculitis, etc.)
Hemorrhagic endovasculitis
Type of vessel(s) involved (chorionic, major stem, etc.)
Volume of affected tissue (grade)
Associated changes (chronic villitis, placental intravascular thrombi, etc.)
Placental intravascular thrombi
Type of vessel(s) involved (chorionic, major stem, etc.)
Number of involved vessels
Associated features (vasculitis, hemorrhagic endovasculitis, etc.)
Trophoblast degenerative knots
Proportion normal, increased, or decreased relative to gestational age
Diffuse or focal
Associated changes (abnormal villous fibrosis or vascularity, decidual vasculopathy, etc.)
Perivillous fibrin deposition and fibrinoid necrosis
Proportion normal, increased, or decreased relative to gestational age
Diffuse or focal
Associated changes (X-cell proliferation, acute inflammation, villous and decidual pathologic conditions)
Erythroblastosis and villous edema
Proportion of nucleated-anucleate erythrocytes (relative to gestational age)
Proportion of affected villi (edema)
Severity of edema (diameter of affected villi relative to gestational age)
Associated changes (acute or chronic inflammation, decidual vasculopathy, abruptio placentae)

fetomaternal interface at the basal plate. The area of involvement may be very narrow or may involve all but subchorionic areas of placenta. Maternal floor infarction has been reported to be a cause of repeated poor pregnancy outcome[21] and has recently been suggested to be of immunologic origin.[22] Its identification is clinically relevant because of the reported potential for recurrence in subsequent pregnancies.

PERIVILLOUS FIBRIN DEPOSITION. The normal villous parenchyma on cross section is dark red and finely granular. Occasionally, white glistening bands of connective tissue carrying the major branches of the fetal stem vessels may be identified. Perivillous fibrin appears as an irregular tan-brown granular material, which appears to be deposited with irregular outlines, and it is located away from the maternal sruface, which allows this lesion to be differentiated from more infarctions. This process is considered to indicate a local ischemic process. In our experience, focal perivillous fibrin deposition is found in 5% to 10% of normal term placentas.

Microscopic examination (Table II)

Noninflammatory changes of the amnion. The normal histologic appearance of the amnion is usually composed of three elements: a low cuboidal epithelium, a dense acellular connective tissue layer, and a loose connective tissue layer with scattered spindle cells that have elongated nuclei and sparse cytoplasm,[23] which phenotypically are macrophages. In severe and prolonged cases of oligohydramnios, squamous debris is ground into the amnion by fetal movements. This is precisely the histologic appearance of amnion nodosum. In this lesion keratinized squamous debris is present in lumps on the amnion surface. The epithelium is not present below the deposit, but there will be reepithelialization of the surface of the squamous deposit to varying degrees. Amnion nodosum is more commonly seen over the chorionic plate than in the reflected membranes. Not surprisingly, it is a rare lesion outside the setting of renal agenesis or prolonged rupture of membranes with persistent oligohydramnios of at least 1 week's duration.

Examination of the amnion and chorion for the presence of meconium-filled macrophages may also provide information regarding the duration of exposure of the membranes to meconium before delivery. Meconium in amniotic fluid diffuses across the amnion and is phagocytosed by the spindle cells of the deep connective tissue layer. After meconium phagocytosis these cells take on a plump shape more characteristic of a macrophage. It is presumed that the number of meconium-laden cells is related to the volume of meconium diffusing from the amniotic fluid and that the depth of diffusion through the amnion, chorion, or decidua is likely related to the duration of exposure to meconium.

Acute placental inflammation. As discussed above, intraamniotic infection may be suspected by gross examination of the umbilical cord, membranes, and chorionic late. The gross diagnosis of intraamniotic infection, however, requires microscopic examination to confirm gross diagnosis and to provide information as to duration-severity and occasionally type of organism. We routinely examine the following sections: two samples of umbilical cord (one close to the distal end of the cord and one from within 10 cm of the insertion on the chorionic plate), two samples of extraplacental membranes (one from the edge of the site of rupture, when identifiable, and one from a membrane roll ex-

tending from the site of rupture to the placental margin), and at least one sample of chorionic plate including chorionic vessels. This method allows us to answer the following questions: Is there an inflammatory process in the pericervical tissues of the site of rupture? If yes, did the inflammation spread laterally through the decidua and therefore remain extraamniotic, or did the inflammation extend into the amniotic fluid? If an intraamniotic inflammation is evident, is there a fetal response (umbilical vasculitis and/or funisitis)? The relevance of these questions to clinical situations such as premature rupture of membranes, preterm labor and delivery, and fetal jeopardy is clear. Patterns of inflammation within the extraplacental membranes may provide clues as to the pathway of the infectious spread.

A discordance between the maternal and fetal components of the inflammatory response may alert the pathologist to the possible involvement of organisms such as group B streptococcus. Pandey et al.[24] clearly demonstrated that certain women who are chronic carriers of this organism have abnormal humoral and cellular immune responses to the streptococcus. When group B streptococcus is involved in an intraamniotic infection in women with absent immune response, the histologic features would be characterized by absent or minimal maternal inflammatory response (in extraplacental membranes and chorionic plate) and a well-developed fetal response (in umbilical cord and chorionic vessels). In the absence of any defensive maternal participation, the fetus-neonate is at risk for severe infectious complications. Indeed this pattern has been observed in cases of catastrophic early-onset neonatal streptococcal sepsis and is commonly observed in women with several children, all of whom suffered neonatal streptococcal sepsis. While there is controversy as to whether women should have cultures performed and/or be treated for streptococcal colonization, it is our opinion that patients with histologic evidence of an inability to mount an immune response to this organism should be considered as candidates for treatment since they are at high risk for developing neonatal sepsis. Only placental examination will identify this group of patients.

Infarction. Microscopically, these lesions will show frank villous necrosis, involving trophoblast, stroma, and endothelia. Recent lesions may show a striking excess of syncytial knotting and early necrosis of the trophoblast. Villous stromal hemorrhage, a marker of placental hypoxia,[25] may be seen in early lesions. Chronic lesions will show an already established villous necrosis and the presence of "ghost villi." These "ghost villi" appear as pale pink eosinophilic bodies with complete loss of all nuclear detail. These lesions are probably at least 7 days old.

Intervillous thrombosis. Microscopically, thromboses are often very bland lesions, consisting of variable combinations of sheets of erythrocytes and bands of organized fibrin. The proportions of erythrocytes and fibrin will vary with the age of the lesion. Acute lesions appear as sheets of erythrocytes apparently pushing villi aside and distorting the normal architecture of the intervillous space. Chronic lesions show uniform eosinophilic bands of fibrin. Occasionally, chronic villitis may be seen at or near a thrombus. In this situation, one may ask whether the chronic inflammation and villous damage were the stimuli of local coagulation. Hemorrhagic endovasculitis may also be seen in villi nearby or at the edge of a thrombus.

Chronic villitis. Chronic villitis is defined as a chronic inflammatory infiltrate of the placental villi composed of plasma cells, lymphocytes, and histiocytes or macrophages. It is unknown if the villous infiltrate is of maternal origin, fetal origin, or a mixed response. Known congenital TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes simplex) are characteristically associated with chronic villositis,[26] while certain histologic features have been proposed to be "pathognomonic" of certain viral entitities.[27] Viruses are rarely isolated from such placentas. The presence of extensive vascular damage, together with stromal hemosiderosis and calcification, suggests cytomegalovirus, while a massive proliferation of placental Hofbauer cells may be more likely to be associated with congenital syphilis.[28] It has been recently suggested that chronic villitis is also associated with maternal immunopathologic states.[29, 30] Chronic villitis with dense chronic intervillositis may be associated with recurrent poor pregnancy outcome[31] and with absent maternal antipaternal blocking factors. Clinical information regarding parity, intercurrent diseases, and past pregnancy outcome are of paramount importance to the appropriate interpretation of this lesion.

The means by which chronic villitis is associated with intrauterine growth retardation has been proposed to be secondary to viral impairment of fetal cellular divisions leading to decreased cell number and a smaller neonate.[32] This has been used to explain the lack of correlation between the degree of placental compromise (amount of villi inflamed) and the degree of fetal compromise. We have demonstrated an association between increased frequency of chronic villitis and decreased ponderal indices in a series of growth-retarded infants delivered at term. In the same study primiparity and chronic villitis were related, independently of birth weight. Low ponderal index indicates a late onset of intrauterine deprivation. To explain these relationships we must propose that there is a predisposition to third-trimester viral infection among primiparous women or that chronic villitis may indicate the presence of a maternal inflammatory-immunologic response to the fetal

allograft. This inflammatory-immunologic response may lead to fetal compromise by alterations in placental function. The possible role of placental macrophages in these important placental functions is currently under investigation in our laboratory.

A sparse chronic villitis involving only occasional anchoring villi is frequently seen in placentas from uncomplicated term deliveries. We have seen the presence of a dense infiltrate, possibly including plasma cells, involving many if not all of the anchoring villi in preeclampsia, idiopathic recurrent abruptio placentae, intrauterine growth retardation, and preterm delivery. The pattern of spread, from maternal to contiguous fetal tissues, strongly suggests an underlying abnormality in the maternal immunologic adaptation to pregnancy. When villi that are distant from the maternal surface are predominantly involved, one may suspect a hematogenous dissemination of infection, such as congenital viral infection.

We describe the relative volume of affected villi and the distribution of affected villi by using a six-point system. Grade 1 applies to a solitary focus of villitis on any one of the four samples routinely examined. Grade 2 describes two foci of villitis on the same slide or different slides. Grade 3 describes multiple foci on at least two different slides. Higher grades (up to six) are assigned on the basis of subjective assessment of percent of involved villi. In normal term placentas the rate of chronic villositis will be approximately 15%. More than 90% will be grade 1 or grade 2. In uncomplicated term deliveries we have rarely found more severe grades of villitis (grade 3 or 4).

Hemorrhagic endovasculitis. Sander et al.[33] first coined the term *hemorrhagic endovasculitis* to describe a lesion observed in cases of unexplained poor pregnancy outcome referred to the Michigan Placental Tissue Registry. This lesion may affect any or all levels of the placental circulation from chorionic vessels and fetal stem vessels to the capillaries of the terminal villi. In the larger vessels it typically appears as a vasoocclusive process with endothelial damage, fibroblast proliferation with scalloping of the vessel outline, erythrocyte fragmentation, and bleeding within the vessel wall and in the villous stroma. At the capillary level only erythrocyte fragmentation and vascular damage are present. Hemorrhagic endovasculitis was originally suggested to be of immunologic origin. However, some investigators believed that the lesion is secondary to hemodynamic instability within the placenta, as a result of association of this lesion with placental infarction.[25] Our investigations have also shown an association of hemorrhagic endovasculitis with placental infarction, chronic villitis, and placental intravascular thrombi. It is most commonly seen in preeclampsia, stillbirth, and intrauterine growth retardation. We would therefore support the interpretation of hemorrhagic endovasculitis as a lesion most frequently resulting from abnormal placental blood flow.

Placental intravascular thrombi. In the past we rarely observed thrombi within placental chorionic or stem vessels (intravascular thrombi). When placental intravascular thrombi were present, they most often were coincident with severe intraamniotic infection or with chroinic villitis. However, in 1989, approximately 10% of placentas examined in our laboratory demonstrated bland mural thrombi in placental vessels. These observations were more frequent in placentas coming from mothers who use cocaine and/or "crack" cocaine. Cocaine causes an intense vasospasm that results in endothelial damage, platelet aggregation, and thrombus formation.[34] Woods et al. have shown that fetal vessels are very sensitive to the vasoactive effects of cocaine (Woods JR, Plessinger MA, Dolkart L. Effect of cocaine upon fetal umbilical blood flow and placental vascular resistance [Abstract]. In: Proceedings of the Rochester Trophoblast Conference with the European Placenta Group, Rolduc, The Netherlands, September 1988). Chasnoff et al.[35] observed neurologic compromise in infants of mothers who ceased cocaine use during pregnancy. It is possible that cocaine can generate venous thrombi in the placenta, predisposing to a situation of chronic thromboembolism, which could chronically damage the fetal central nervous system. These possibilities deserve further investigation.

Trophoblast degenerative knots. In degenerative knots the syncytial nuclei are dark blue-black and pyknotic. Fox[36] has studied the formation of such knots in vitro, and has shown that they develop as early as 24 hours after culture in a low oxygen tension environment. From the clinical point of view these lesions are diffusely increased in situations of chronic uterine vascular insufficiency (e.g., hypertensive diseases of pregnancy) and focally increased in acute abruptio placentae.

Perivillous fibrin deposition and fibrinoid necrosis. These are discussed together as they appear somewhat similar histologically and may coexist in the same placentas. Microscopically, in perivillous fibrin deposition, eosinophilic fibrin coats the surfaces of villi, often matting them together. The villi themselves remain viable, although there may be necrosis of syncytiotrophoblast. Fibrinoid necrosis is an acellular mass of dense eosinophilic material that often appears as a lump on the trophoblast basement membrane and is covered completely or partially by syncytiotrophoblast epithelium. As the mass expands, adjacent villi may become matted to the lesion. As a final process it is thought to replace the villous stroma. Clinically, both these processes are seen more frequently and more extensively in term or postterm placentas. Excess perivillous fibrin deposition may be seen in preterm placentas in association with severe intraamniotic infection. Intrauterine infection

may compromise the fetus in many ways besides the risks of sepsis. We have observed alterations in fetal heart rate, biophysical profile, and umbilical blood flow in association with intraamniotic infection. If infection leads to direct villous damage, nutrient transfer and gas exchange may be impaired.

Erythroblastosis and villous edema. In the adult 24 to 48 hours is required for the bone marrow to respond to hematopoietic stress by production of nucleated erythrocytes.[37] The presence of excess nucleated erythrocytes in placental circulation suggests a duration of stress for a minimum of 24 hours. We have observed excess nucleated erythrocytes only in the presence of placental histologic features that are more suggestive of chronic rather than acute insults. Intrauterine growth retardation and preeclampsia are examples of chronic processes that are often associated with excess production of nucleated erythrocytes.[38] In stillbirths the presence of nucleated erythrocytes suggests a more chronic stress and would speak against an acute intrapartum event as being the cause of the fetal damage.

An excess of nucleated erythrocytes in the placental circulation generally accompanies both immune and nonimmune hydrops, since both involve decreased tissue oxygen delivery and increased bone marrow activity. The excess release of immature nucleated erythrocytes from the marrow may be a response to accelerated erythrocyte destruction, as in Rh isoimmunization, or to bone marrow effects, as in congenital parvovirus infections.[39]

Erythroblastosis and villous edema are discussed together because they frequently coincide in diseases such as immune and nonimmune hydrops. In these situations the villous swelling mirrors the diffuse fetal edema. Fetoplacental edema has been associated with decreased red blood cell mass and decreased hepatic production of proteins leading to decreased intravascular oncotic pressure.[40]

Villous edema may be observed in several situations besides hydrops fetalis. Naeye et al.[41] reported the association of villous edema with acute intraamniotic infection. They suggested that villous swelling might result in decreased oxygen delivery to the fetus and fetal distress. We suggest that villous edema may be the result of inflammatory cytokines in the intervillous space, altering trophoblast and capillary permeability. It is possible that different bacteria may have different capacities to generate inflammatory cytokines and prostanoids (e.g., endotoxin-producing *Escherichia coli*). Further studies of the roles of inflammatory mediators in villous damage are necessary.

Acute and chronic fetal insults: The role of placental pathologic examination

The pathologist is trained to ask two questions: "Where is the lesion?" and "How long has it been there?" From the medicolegal point of view it is frequently assumed that the lesion was caused to a previously intact fetus during the course of labor and delivery. However, the fallacy of this reasoning is only beginning to become clear to the medical community. We must communicate this information to our patients. For better or worse the American society is one with high expectations, and a perfect baby is certainly among them. We value "fair play" and "justice." When the unexpected happens, American society looks for someone to be held accountable, because to have a damaged baby somehow "isn't fair." We certainly agree with this but suggest that the only way to prevent fetal and neonatal damage is to better understand the causes of such damage. Mann[42] has states that "At least half, if not more, of all brain-damaged children have no scientifically or clinically based explanation for their problem." Not more than 10% of severe mental retardation is considered to be attributable to perinatal causes.[43, 44]

Clearly, the search in the intrapartum period for the roots of every case of neurologic damage is not rewarding. This attitude toward the significance of intrapartum events and management stemmed in part from the belief that the intrauterine environment was the perfect nurturant environment for the duration of gestation, buffering the fetus from all hazards and stresses. Only on departure from this "nirvana" could the fetus be damaged. However, as the methods of antepartum fetal surveillance become more sensitive, it is becoming more evident that this is untrue. Bejar et al.[45] have described antepartum cerebral infarction linked to intraamniotic infection. It is recognized that clinical chorioamnionitis is a late stage in the process. The majority of cases of histologically evident intraamniotic infection, with umbilical vasculitis used as a marker, are clinically silent. Chasnoff et al.[35] linked maternal cocaine use to cerebral infarction. Their work[35] also suggests that cocaine use in early pregnancy may have lasting effects on the developing fetal central nervous system. In addition, we have observed the presence of placental thrombi in association with maternal cocaine use, which may possibly cause neurologic damage by acting as a source of thromboemboli. While it is recognized that congenital viral infection is associated with many forms of neurologic damage, most maternal infections are clinically silent. Therefore it is not surprising that in the overwhelming majority of cases of chronic villitis no antecedent maternal illness can be documented. These cases will be detected only where placental examination is routinely performed. It is a clinical truth that there are cases of preeclampsia in which maternal disease is severe and the fetus and placenta are normal and also cases of intrauterine fetal death associated with what clinically seems to be mild preeclampsia.

Robertson et al.[45a] have confirmed that the uterine

vascular anatomy of preeclampsia may occur in the absence of maternal signs and symptoms. We have also observed similarities in placental pathologic conditions between idiopathic intrauterine growth retardation and intrauterine growth retardation associated with preeclampsia. It may be that idiopathic intrauterine growth retardation is one form of preeclampsia that specifically targets the fetoplacental unit, sparing other maternal organ systems. We propose that processes linked to long-term compromise of neurologic function are usually clinically silent. These processes are more likely to be detected in routine gross and microscopic examination of the placenta.

The duration of fetal compromise is very important in regard to the likelihood of long-term neurologic damage. Myers' work[46] has shown that short exposure to complete asphyxia will most likely result in no long-term neurologic sequelae, while prolonged partial asphyxia will most frequently result in neurologic damage in a histologic distribution consistent with that observed in human cases of cerebral palsy. Clapp et al.[47] have shown cerebral damage and intermittent interference with the umbilical circulation. An understanding of the relative time-frame associated with the different placental processes is helpful. Acute ascending infection is generally a short-term process. In the presence of acute ascending infection the mother will go into labor soon or will become clinically infected, and the infant will be delivered. The life span of the adult polymorphonuclear leukocyte is approximately 2 to 3 days in tissue culture.[48] If fragmentation of polymorphonuclear leukocytes is present, one may suggest that the inflammatory event was initiated at least 2 days before delivery. Infiltrates with numerous or degenerating polymorphonuclear leukocytes will likely represent even longer durations and/or severity of inflammation. Trophoblast degenerative knots may develop as quickly as 24 hours after in vitro exposure to lowered oxygen tension. However, trophoblast knots are also common in situations of chronic uterine vascular insufficiency, such as preeclampsia. Trophoblast knots that develop as a response to an acute stimulus will be associated with normal-appearing villi. Trophoblast knots appearing as part of a more chronic process will have abnormal villous structure, including fibrosis and hypovascularity. Infarctions with loss of nuclear detail will likely be >7 days old. The "age" of chronic villitis may be a more complicated issue. The premature placenta, with its functionally immature macrophages, may be less capable of demonstrating a cellular infiltrate than a term placenta. Small foci of villitis in a preterm placenta may represent a significantly more severe and protracted inflammation than in a term placenta. We must also consider the possibility that inflammation may present in a different fashion in the very immature placenta. In the very immature placenta a cellular infiltrate may not develop. Instead, edema, a cardinal sign of inflammation, may be the only histologic evidence of inflammation. Therefore the gestational age of the placenta is critical to interpreting the presence of chronic inflammation.

Table III summarizes the approximate timing of the most common placental lesions. The relevance of these concerns to medicolegal issues is obvious. If a process capable of damaging the fetus can "more likely than not" be said to predate a patient's coming to the labor and delivery suite, the role of intrapartum obstetric care in the genesis of neurologic damage should be in serous doubt.

The need for placental pathologic examinations in all pregnancies

At present there is no cure for neurologic damage except the early detection of infants at risk for long-term compromise, permitting early intervention with remedial programs to optimize neurologic development. Examination of the placenta may provide the only source of evidence that such risk factors exist in a clinically "normal" pregnancy. Many investigations suggest that the consideration of placental lesions will provide information that is useful to both parents and pediatricians. Chronic villitis, for example, is associated with growth retardation in idiopathic preterm deliveries but not in preterm preeclampsia. Chronic villitis has been linked to congenital viral infection[26] but also has been suggested to reflect maternal-fetal immunopathologic conditions.[29, 30] We have observed a significant increase in chronic villitis among primiparous women, independent of other maternal factors (Salafia CM, Vintzileos AM, Silberman L. Placental pathology of idiopathic intrauterine growth retardation at term. To be published, 1990). The venous or arterial nature of placental thrombi, the presence or absence of intraamniotic infection, and all relevant clinical data must be considered together to assess the risk to the infant. Children and parents deserve better than a situation where "At least half if not more of children with neurologic damage have no scientifically or clinically based explanation for their problem."[42] Feelings of frustration and helplessness naturally accompany the loss of parents' dreams for their offspring when death or neurologic compromise of their child occurs. Parents will look for a "reason" for the unexpected bad outcome and, failing to find one, they will turn on themselves ("What did I do wrong?") or their care-givers ("What did my obstetrician do wrong?"). In many of these cases examination of the placenta is the only avenue to detect the clinically silent, and often untreatable, culprit. Table IV summarizes the possible causes of the most common placental lesions. Moreover, if the clinically

Table III. Approximate timing of placental lesions

Acute inflammation	Timing depends on depth of leukocyte migration (across membranes and chorionic plate) and severity of stimulus (type of organism and size of inoculum). May also vary with host response. If no degenerated leukocytes are present, stimulus likely to be present <48 hr. Presence and extent of leukocyte degeneration imply longer duration
Amnion nodosum	At least 1 wk of severe oligohydramnios
Meconium histiocytosis	At least 1 to 6 hr exposure to meconium. Deeper diffusion (into chorion) implies longer duration
Infarction	If no nuclear detail, at least 1 wk old. In lesions with nuclear detail (<1 wk old) degree of trophoblast and stromal preservation implies a greater or lesser age
Abruptio placentae	Dated by adjacent villous changes (see Infarction above)
Intervillous thrombosis	Age varies with maternal and fetal blood types, volume of clot, and regional flow characteristics
Chronic villitis	Days to months
Placental intravascular thrombi	Days to months
Maternal floor infarction	Weeks to months

Table IV. Possible causes of placental lesions

Acute inflammation	Acute ascending chorion-decidua, amnion, infection of vaginal chorionic plate, chorionic vessel; organisms such as umbilical cord bacteria, yeast, mycoplasma (rarely the result of hematogenous spread of maternal sepsis, e.g., listeriosis, streptococcus)
Infarction, intervillous thrombosis	Decidual vasculopathy (related to preeclampsia or preexisting maternal medical disease), maternal cocaine use, May occur near margins of normal placentas
Abruptio placentae	Decidual vasculopathy (related to preeclampsia or preexisting maternal medical disease), maternal cocaine use. More frequent in acute inflammation
Maternal floor infarction	? Maternal-fetal immunopathologic condition, may be recurrent
Chronic villitis	Congenital infection (e.g., TORCH). Maternal-fetal immunopathologic condition, especially with history of recurrent poor pregnancy outcome
Hemorrhagic endovasculitis	Circulatory instability (e.g., preeclampsia, stillbirth). Often associated with other placental lesions (e.g., infarction, thrombosis, chronic villitis)
Erythroblastosis and edema	Immune and "nonimmune" hydrops, congenital viral infection, acute inflammation. Erythroblastosis may be seen in intrauterine growth retardation, preeclampsia, or antiphospholipid antibody disease with massive placental infarction
Placental intravascular thrombi	Associated with other placental lesions (infarction, chronic villitis hemorrhagic endovasculitis). Possible association with maternal cocaine use
Meconium histiocytosis	Significance varies with gestational age. At term may be insignificant. May be seen with fetal distress
Amnion nodosum	Chronic decrease in amniotic fluid volume

silent problem detected by placental examination is treatable, subsequent pregnancy outcome may be improved. On the basis of placental gross and/or microscopic findings, we have suggested that there are certain conditions that are well treatable but all have a high risk of recurrent poor outcome if not treated. These conditions include abnormal immunity to group B streptococcus, presence of maternal antiphospholipid antibody, lack of antipaternal blocking factors, and decidual vasculitis with positive antinuclear antibodies in the absence of clinical lupus erythematosus. Several of these patients have subsequently had their first uncomplicated pregnancy after treatment and appropriate surveillance. Since most long-term neurologic compromise will not be detectable until after 1 year of age, routine examination of all placentas would appear to be the only way to consistently provide these vital data.

Cost-effectiveness of routine placental pathologic examination

Defense lawyers and insurance companies have recognized the potential value of placental examination in medicolegal cases. Some investigators have suggested that sections from all placentas should be saved or that

paraffin blocks be prepared but slides not be cut or examined until the information is needed. We feel that this is a failure to see the broad implications of placental pathologic examination. Space in most hospitals is at a premium, and significant space is required to store placentas or even placental blocks. How long should a placenta be saved? A healthy infant at birth or at hospital discharge at 3 days of age does not guarantee a healthy, intact child. Many forms of neurologic compromise resulting from antepartum fetal insults may not manifest themselves until months after birth.

In many states that average award for medical negligence in cases of cerebral palsy is $5 to $6 million and the average settlement award is $1.5 million. The costs of placental examination in Connecticut average $225. The busy obstetricians delivers approximately 300 infants a year. Over 30 years of practice, he/she will deliver approximately 9000 infants. The total cost generated by routine examination of all these placentas would be approximately $2 million. This generated cost is much less than the average award for medical malpractice of $5 to $6 million and worthwhile to avoid one case of alleged medical malpractice. We feel that placental examination should occur at delivery and not after problems arise in the early years of life. We therefore suggest that routine examination of the placenta should be as much a part of normal care in pregnancy as the assignment of Apgar scores.

Comment

In cases of poor neonatal outcome, examination of the placenta may not only identify the exact cause but also give an indication of the time-frame characteristics of the insult. This information will lead to clearer understanding of the origin of the abnormal neurologic outcome (e.g., infectious or decidual vascular) and also to appropriate determination of medicolegal responsibilities for the poor outcome. The benefits of placental examination in cases of poor neonatal outcome are well recognized in the literature.

It is our opinion that the significant benefits of routine placental examination for all deliveries have not been recognized. Routine placental examination is the only tool that we can possibly use to better understand causes of intrauterine neurologic insult. Without this understanding we cannot develop methods to prevent or treat those clinically silent intrauterine processes. It is time that the medical community starts thinking about effective screening methods to permit early identification of the 80% of neurologically damaged children who have no history of peripartum distress. At the same time this approach will figure prominently in medicolegal defense.

REFERENCES

1. Fox H. Pathology of the placenta. In: Bennington JL, ed. Major problems in pathology. Philadelphia: WB Saunders, 1978 vol 7.
2. Benirschke K, Driscoll SG. The pathology of the human placenta. New York: Springer-Verlag, 1967:129.
3. Kaplan C. Placental pathology in perinatal diagnosis. In: Sciarra JJ, ed. Maternal and fetal medicine. Philadelphia: JB Lippincott, 1980:1-20.
4. Walker CW, Pye BG. The length of the human umbilical cord: a statistical report. Br Med J 1960;1:546-8.
5. Malpas P. Length of the human umbilical cord at term. Br Med J 1964;1:673-4.
6. Purola E. The length and insertion of the umbilical cord. Ann Chir Gynaecol 1968;57:621-5.
7. Gardiner JP. The umbilical cord: normal length, length in cord complications, etiology and frequency of coiling. Surg Gynecol Obstet 1922;34:252-8.
8. Moessinger AC. Fetal lung growth in experimental uteroabdominal pregnancy. Obstet Gynecol 1986;68:675-8.
9. Ottolenghi-Preti GF, Bailo P. I funicoli lunghi (studio clinicostatistico su quasi 30,000 parti). Ann Obstet Ginecol 1950;72:293-9.
10. Swanberg H, Wiqvist N. Rupture of the umbilical cord during pregnancy: a report of a case. Acta Obstet Gynecol Scand 1951;30:203-6.
11. Perrin EVDK, ed. Pathology of the placenta. New York: Churchill Livingstone, 1984:17.
12. Altshuler G, Tsang RC, Ermocilla R. Single umbilical artery: correlation of clinical status with umbilical cord histology. Am J Dis Child 1975;129:697-700.
13. Driscoll SG. Umbilical cord teratoma. Hum Pathol 1985;16:1178-81.
14. Smith D, Majmudar B. Teratoma of the umbilical cord. Hum Pathol 1985;16:190-3.
15. Reynolds SRM. The physical forces of blood flow in the umbilical vein: a study in functional anatomy. In: Langot LD, Rencan DD, eds. Fetal and newborn cardiovascular physiology: Fetal and newborn circulation. New York: Garland STPM Press. 1978 vol 2:47-67.
16. Robinson LK, Jones KL, Benirschke K. The nature of structural defects associated with velamentous and marginal insertion of the umbilical cord. Am J Obstet Gynecol 1983;146:191-3.
17. Christianson RE. Gross differences observed in the placentas of smokers and nonsmokers. Am J Epidemiol 1979;110:178-87.
18. Leuschen MP, Davis RB, Boyd D, Goodlin RC. Comparative evaluation of antepartum and postpartum platelet function in smokers and nonsmokers. Am J Obstet Gynecol 1986;155:1276-80.
19. Batcup G, Tovey LAD, Longster G. Fetomaternal blood group incompatibility studies in placental intervillous thrombosis. Placenta 1983;4:449-54.
20. DeCosta EJ, Gerbie AB, Andresen RH, Gallanis TC. Placental tumors: hemangiomas. Obstet Gynecol 1956;7:249-59.
21. Khudr G, Soma H, Benirschke K. Trophoblastic origin of the X cell and the placental site giant cell. Am J Obstet Gynecol 1973;115:530-5.
22. Maddox DE, Kephart GM, Coulam CB, Butterfield JH, Benirschke K, Gleich GJ. Location of a molecule immunochemically similar to eosinophil major basic protein in human placenta. J Exp Med 1984;160:29-41.
23. Bourne G. The human and amnion and chorion. London: Lloyd-Luke, 1962 vol 15:4-8.
24. Pandey JP, Baker CJ, Kasper DL, Fudenberg HH. Two unlinked genetic loci interact to control the human immune response to type III group B streptococcal antigen. J Immunogenet 1984;11:159-63.

25. Fox H, Herd ME, Harilal KR. Morphological changes in the placenta and decidua after induction of abortion by extra-amniotic prostaglandin. Histopathology 1978;2: 145-51.
26. Driscoll SG. Histopathology of gestational rubella. Am J Dis Child 1969;118:49-53.
27. Blanc WA. Pathology of the placenta and cord in ascending and in hematogenous infection. In: Perinatal infections. Ciba Foundation Symposium 77 (new series). Amsterdam: Excerpta Medica, 1980:32.
28. Russell P, Altshuler G. Placental abnormalities of congenital syphilis: a neglected aid to diagnosis. Am J Dis Child 1974;128:160-3.
29. Labarrere C, Manni J, Salas P, Althabe O. Intrauterine growth retardation of unknown etiology. I. Serum complement and circulating immune complexes in mothers and infants. Am J Reprod Immunol Microbiol 1985;8:87-93.
30. Labarrere C, Althabe O, Caletti E, Muscolo D. Deficiency of blocking factors in intrauterine growth retardation and its relationships with chronic villitis. Am J Reprod Immunol Microbiol 1986;10:14-19.
31. Redline RW, Abramowsky CR. Clinical and pathologic aspects of recurrent placental villitis. Hum Pathol 1985;16:727-31.
32. Naeye RL, Tafari N. Risk factors in pregnancy. In: Naeye RL, ed. Diseases of the fetus and newborn. Baltimore: Williams & Wilkins, 1983:120.
33. Sander CH, Kinnane L, Stevens NG, Echt R. Haemorrhagic endovasculitis of the placenta: a review with clinical correlation. Placenta 1986;7:551-74.
34. Simpson RW, Edwards WD. Pathogenesis of cocaine-induced ischemic heart disease: autopsy findings in a 21-year-old man. Arch Pathol Lab Med 1986;110:479-84.
35. Chasnoff IJ, Bussey ME, Savich R, Stack CM. Clinical and laboratory observations: perinatal cerebral infarction and maternal cocaine use. J Pediatr 1986;108:456-61.
36. Fox H. The significance of villous syncytial knots in the human placenta. J Obstet Gynaecol Br Commonw 1965;71:347-55.
37. Salafia CM, Weigl CA, Foye GJ. Correlation of placental erythrocyte morphology and gestational age. Pediatr Pathol 1988;8:495-502.
38. Altshuler G, Herman AA. The medicolegal imperative: placental pathology and epidemiology. In: Stevenson DK, Sunshine P, eds. Fetal and neonatal brain injury: mechanisms, management and risk of practice, Philadelphia: BC Decker, 1989:250-63.
39. Maeda H, Shimokawa H, Satoh S, Nakano H, Nunoue T. Nonimmunologic hydrops fetalis resulting from intrauterine human parvovirus B-19 infection: report of two cases. Obstet Gynecol 1988;72:482-5.
40. Nicolaides KH, Warenski JC, Rodeck CH. The relationship of fetal plasma protein concentration and hemoglobin level to the development of hydrops in rhesus isoimmunization. AM J OBSTET GYNECOL 1985;152:341-4.
41. Naeye RL, Maisels J, Lorenz RP, Botti JJ. The clinical significance of placental villous edema. Pediatrics 1983;71:588-94.
42. Mann LI. Pregnancy events and brain damage. AM J OBSTET GYNECOL 1986;155:6-9.
43. Rosen MG, Hobel CJ. Prenatal and perinatal factors associated with brain disorders. Obstet Gynecol 1986;68: 416-21.
44. Perkins RP. Perspectives on perinatal brain damage. Obstet Gynecol 1987;69:807-19.
45. Bejar R, Wozniak P, Allard M, et al. Antenatal origin of neurologic damage in newborn infants. I. Preterm infants. AM J OBSTET GYNECOL 1988;159:357-63.
45a. Robertson WB, Brosens I, Dixon HG. The pathological response of the vessels of the placental bed to hypertensive pregnancy. Pathol Bacteriol 1967;93:581-92.
46. Myers RE. Two patterns of perinatal brain damage and their conditions of occurrence. AM J OBSTET GYNECOL 1972;112:246-77.
47. Clapp JF, Mann LI, Peress NS, Szeto HH. Neuropathology in the chronic fetal lamb preparation: structure-function correlates under different environmental conditions. AM J OBSTET GYNECOL 1981;141:973-84.
48. Golde DW. Production, distribution, and fate of neutrophils. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA, eds. Hematology. 3rd ed. New York: McGraw-Hill, 1983:763.

Role of vasopressin in mediation of fetal cardiovascular responses to acute hypoxia

Kathleen M. Piacquadio, MD, Robert A. Brace, PhD, and Cecilia Y. Cheung, PhD
La Jolla, California

The present study was designed to test the hypothesis that arginine vasopressin mediates the fetal cardiovascular response to acute hypoxia. Chronically catheterized sheep fetuses at 126 to 138 days' gestation were infused with either an arginine vasopressin pressor antagonist (n = 8) or saline solution as control (n = 8). A 30-minute hypoxia was induced by infusion of nitrogen containing 5% carbon dioxide into the maternal trachea. Fetal arterial Po_2 decreased 13.1 ± 1.3 (SE) mm Hg from a basal value of 23.8 ± 1.5 mm Hg and there was no significant difference in the degree of hypoxia between the two groups. Fetal arterial and venous pressures increased significantly, whereas the blood volume decreased, but these changes were similar between the control and arginine vasopressin–blocked fetuses. Heart rate fell similarly in both groups during hypoxia by an average of 35 beats/min. At the termination of hypoxia, heart rate in the blocked group rebounded to levels significantly above baseline and remained elevated for 30 minutes, whereas heart rate in the control group returned slowly to basal values. Recovery of arterial pressure, venous pressure, and blood volume were similar in the two groups. Thus it appears that arginine vasopressin may mediate in part the fetal heart rate response to acute hypoxia. However, the blood volume, arterial pressure, and venous pressure responses to hypoxia appear to be induced by factors other than arginine vasopressin. (Am J Obstet Gynecol 1990;163:1294-300.)

Key words: Fetus, heart rate, arterial pressure, venous pressure, blood volume, arginine vasopressin

Moderate to severe acute hypoxia in the ovine fetus is associated with increases in arterial and venous pressures but decreases in heart rate and blood volume.[1-4] These cardiovascular changes have been shown to be mediated at least in part by circulating catecholamines.[2, 5-9] However, after α-adrenergic plus β-adrenergic receptor blockade in the fetal sheep, the rise in vascular pressures during hypoxia is attenuated and delayed but not abolished,[10] suggesting that in addition to the catecholamines other factors may be responsible for the mediation of the cardiovascular changes in response to hypoxia. In view of the fact that large increases in fetal plasma arginine vasopressin concentrations occur during severe hypoxia[4, 11] and that arginine vasopressin is a potent vasoconstrictor that, when infused into the fetus, produced arterial and venous hypertension and bradycardia,[12-14] it is possible that arginine vasopressin may mediate some of the cardiovascular responses to hypoxia in the fetus. Previously we reported that in association with the hypertension and bradycardia, fetal blood volume decreases during both mild and severe hypoxia.[10] This fall in blood volume during mild hypoxia is mediated by the catecholamines. However, during severe hypoxia the blood volume changes can no longer be reversed by α-adrenergic plus β-adrenergic receptor blockade. Because fetal plasma arginine vasopressin levels increase during acute hypoxia when Po_2 falls below 17 mm Hg,[15, 16] arginine vasopressin could play a role in mediating the decrease in fetal blood volume during severe hypoxia. The present study was designed to test the hypothesis that the arterial and venous hypertension and bradycardia that occur during acute severe hypoxia are mediated by arginine vasopressin. In addition, the possibility that arginine vasopressin may induce the fetal blood volume response to severe hypoxia was investigated. For these studies, the fetal cardiovascular V_1 receptors were blocked by the use of the pressor antagonist $d(CH_2)_5-Tyr(Me)$ arginine vasopressin. (Peninsula Laboratories, Belmont, Calif.)

From the Division of Perinatal Medicine, Department of Reproductive Medicine T-002, University of California, San Diego.
Supported by National Institutes of Health grants HD20295 and HD20299.
Received for publication August 23, 1989; revised February 8, 1990; accepted May 1, 1990.
Reprint requests: Cecilia Y. Cheung, PhD, Department of Reproductive Medicine T-002, University of California, San Diego, La Jolla, CA 92093.
6/1/22093

Methods

Animal preparations. Eleven time-dated pregnant ewes with singleton fetuses at an average gestational age of 131 ± 1 (SE) days (Nebeker Ranch, Lancaster, Calif.) were used for the study. After anesthesia was induced with administration of halothane to the ewe, catheters were placed in the fetal tibial arteries and saphenous veins, and the tips were advanced to the level of the diaphragm to rest in the descending aorta and inferior vena cava, respectively.[17] Two additional catheters were placed in the amniotic cavity. An indwelling catheter was placed in the maternal femoral vein. All catheters were tunneled subcutaneously to a nylon pouch sewn to the flank of the ewe. A nonocclusive catheter was placed in the maternal trachea through a small midline incision in the neck.[18] Prophylactic antibiotics, consisting of 800,000 U penicillin G plus dihydrostreptomycin, Pfizer, New York) intramuscularly to the ewe and 1 gm ampicillin (Omnipen, Wyeth Laboratories, Inc., Philadelphia) into the amniotic fluid, were administered on the first 5 postoperative days and after each experimental session. Vascular catheters were flushed daily with heparin sodium solution (1000 U/ml).

Experimental protocol and measurements. The first experiments were conducted at least 5 days after surgery when the gestational age of the fetuses was 132 ± 1 (SE) days. In five animals, a second experiment was performed 2 days after the first. The animals subjected to two experiments were randomly assigned to either the arginine vasopressin antagonist or control group for their first experiment and to the opposite group for their second experiment. The experimental protocol consisted of a 30-minute control period and a 30-minute period of hypoxia, followed by a 3-hour recovery period. Hypoxia was induced by the infusion of nitrogen containing 5% carbon dioxide into the trachea of the ewe while the ewe was freely breathing room air. In fetuses subjected to arginine vasopressin blockade, the pressor antagonist $d(CH_2)_5$ −Tyr(Me) arginine vasopressin was administered intravenously as a 25 μg bolus in 3 ml 0.9% sodium chloride at the start of the experiment. This was followed by a continuous infusion of the arginine vasopressin analog at 1 μg/min (in 0.39 ml/min of isotonic saline solution) for the duration of the experiment. Control fetuses received a 3 ml bolus of 0.9% sodium chloride followed by a continuous infusion of 0.9% sodium chloride at a similar rate. Preliminary studies were performed to ensure the effectiveness of blockade for the pressor effects of arginine vasopressin. With the same dose of the arginine vasopressin antagonist as in the present study, the increase in fetal arterial pressure in response to an intravenous 30 ng bolus of arginine vasopressin was abolished. On the other hand, when given alone the antagonist had no effect on arterial or venous pressures or heart rate.

Fetal arterial pressure, venous pressure, heart rate, and amniotic fluid pressure were continuously monitored on a polygraph recorder and mean values were stored at 30-second intervals with an on-line computer as previously described.[17] Amniotic fluid pressure was used as the zero pressure reference for fetal vascular pressures. Fetal arterial blood (4 ml) was sampled at 15-minute intervals for the first 2 hours of the experiment and at 1 hour intervals for the last 2 hours. The volume of each blood sample removed was replaced with an equivalent amount of maternal blood to prevent a sampling-induced decrease in hematocrit. Three milliliters of blood was placed in each chilled heparinized tube containing aprotinin (Trasylol, Sigma Chemical, St. Louis, Mo.) at 500 KIU/ml and then centrifuged. The plasma was stored at −70° C until used for hormone concentration measurements. One milliliter of blood was used to measure blood gas and pH values (IL1302 blood gas analyzer at 39.5° C) as well as microhematocrit in triplicate as previously described.[19, 20] Blood volume as a fraction of that before the hypoxia was calculated as the mean hematocrit during the prehypoxic period divided by the hematocrit of each sample. This method has been shown to be valid.[19, 20] In addition, because the hematocrit values during the control period in the control and arginine vasopressin–blocked fetuses were similar (Table I), arginine vasopressin did not appear to have significant effects on hematocrit measurements.

Radioimmunoassay. Plasma arginine vasopressin concentrations were determined by a double antibody radioimmunoassay procedure with antiserum generously provided by Dr. Benjamin U (University of California, San Diego, Calif.). The plasma samples were extracted with C18 microcolumns (Sep Pak, Waters Associates, Milford, Mass.) as described previously.[21] The extraction efficiency as determined by the addition of 50 pg arginine vasopressin standard to 1 ml of plasma was 92.4% ± 4.7%. The intraassay and interassay coefficients of variation for the assay were 5.6% and 16.0%, respectively. The minimum amount of arginine vasopressin detectable was 0.2 pg per tube. Arginine vasopressin measurements were not performed in the fetuses that received the antagonist because this compound cross-reacted 100% with the arginine vasopressin antiserum.

Data analysis. The data are expressed as the mean ± SE. A p value of <0.05 was considered statistically significant. Potential differences in mean values in the control and antagonist-treated fetuses during the control period before hypoxia were analyzed with an unpaired Student t test. To determine whether there were

Table I. Control cardiovascular and arterial blood gas variables in ovine fetuses at 126 to 133 days' gestation

Variable	*Controls**	*Arginine vasopressin blocked**
Arterial pressure (mm Hg)	45.7 ± 2.2	44.3 ± 2.9
Venous pressure (mm Hg)	4.2 ± 0.2	4.1 ± 0.6
Heart rate (beats/min)	175 ± 4	167 ± 8
Oxygen tension (mm Hg)	21.4 ± 1.2	24.5 ± 1.3
Carbon dioxide tension (mm Hg)	53.2 ± 1.8	53.9 ± 1.5
pH	7.33 ± 0.01	7.32 ± 0.02
Hematocrit (%)	33.4 ± 2.9	30.9 ± 1.5
Plasma arginine vasopressin (pg/ml)	4.8 ± 0.8	†

Values are presented as mean ± SE.
*$N = 8$.
†Arginine vasopressin concentrations were not measured in this group.

statistically significant changes with time in one variable, a two-way analysis of variance for repeated measurements was used. To determine whether the changes with time in the control group were different from those in the arginine vasopressin–blocked group, a two-way analysis of variance for replication and repeated measurements was used. The relationship between two variables at a given time was analyzed with bivariate regression analysis methods.

Results

In ovine fetuses at 126 to 133 days' gestation, mean vascular pressures, heart rate, blood gas values, and plasma arginine vasopressin concentration during the 30-minute control period in the groups treated with arginine vasopressin antagonist and saline solution are shown in Table I. There were no significant differences in these variables between the two groups. These values are consistent with those obtained in our laboratory.

The changes in fetal arterial blood gas and pH values during hypoxia in the arginine vasopressin–blocked and control fetuses are shown in Fig. 1. In the control fetuses, P_{O_2} decreased by 9.9 ± 1.4 mm Hg to 11.5 ± 0.8 mm Hg in response to hypoxia, whereas in the arginine vasopressin–blocked fetuses, the P_{O_2} fell by 12.8 ± 1.2 mm Hg to 11.8 ± 0.8 mm Hg. Arterial P_{O_2} returned to basal levels immediately after the termination of hypoxia. There was no significant difference in the degree of hypoxia achieved in the two groups. During hypoxia the fetal arterial pH and P_{CO_2} changes were similar in the two groups (Fig. 1); arterial pH did not change, whereas P_{CO_2} fell significantly. Immediately after the hypoxic episode, pH decreased, whereas P_{CO_2} increased significantly but returned to baseline within 60 minutes.

The changes in arterial pressure, venous pressure, and heart rate during hypoxia in the control and arginine vasopressin–blocked fetuses are shown in Fig. 2. Arterial pressure increased during hypoxia ($p < 0.0001$) in the fetuses treated with saline solution, and these changes were not significantly altered by the arginine vasopressin blockade. In both groups, arterial pressure decreased immediately after the hypoxia, followed by a secondary rise to levels above the control, and returned to baseline by the second hour of the recovery period. Venous pressure increased during hypoxia ($p < 0.0001$) and immediately returned to basal levels after hypoxia. The venous pressure responses were not different in the two groups.

During hypoxia, heart rate fell similarly in both groups of fetuses by an average of 35 beats/min ($p < 0.0001$). In the immediate posthypoxic period, heart rate in the arginine vasopressin–blocked fetuses rapidly increased to levels significantly higher than baseline. Heart rate in the control fetuses remained depressed and gradually returned to basal levels at 30 minutes after hypoxia. These changes in fetal heart rate during the recovery period were different between the two groups ($p < 0.01$).

Fetal blood volume during hypoxia decreased by 8.0% ± 1.0% and 10.8% ± 1.3% in the control and arginine vasopressin–blocked groups, respectively (Fig. 3). There was no significant difference in the fall in blood volume between the two groups. With bivariate regression analysis, a significant ($p < 0.006$) linear relationship was found between the decrease in blood volume and the fall in P_{O_2} in the two groups at the end of hypoxia (Fig. 4).

Fig. 5 shows the increase in plasma arginine vasopressin concentrations in control fetuses during and after the hypoxic period. Plasma arginine vasopressin concentrations increased from a mean basal value of 4.8 ± 0.8 pg/ml to 60.6 ± 27.1 pg/ml at the end of the 30-minute hypoxic period ($p < 0.001$). Arginine vasopressin levels gradually returned to baseline within 30 minutes after the end of hypoxia.

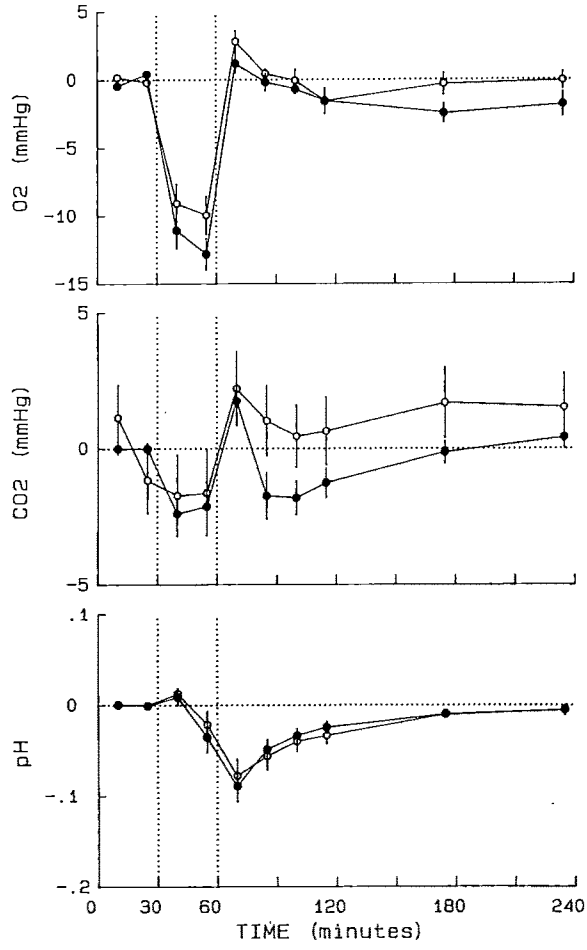


Fig. 1. Fetal arterial blood gas tension and pH changes (mean ± SE) during control, hypoxic, and recovery periods in controls *(open circles)* and arginine vasopressin–blocked *(closed circles)* ovine fetuses. Data are expressed as change from mean control value during the first 30 minutes. *Vertical dotted lines* indicate beginning and end of hypoxia.

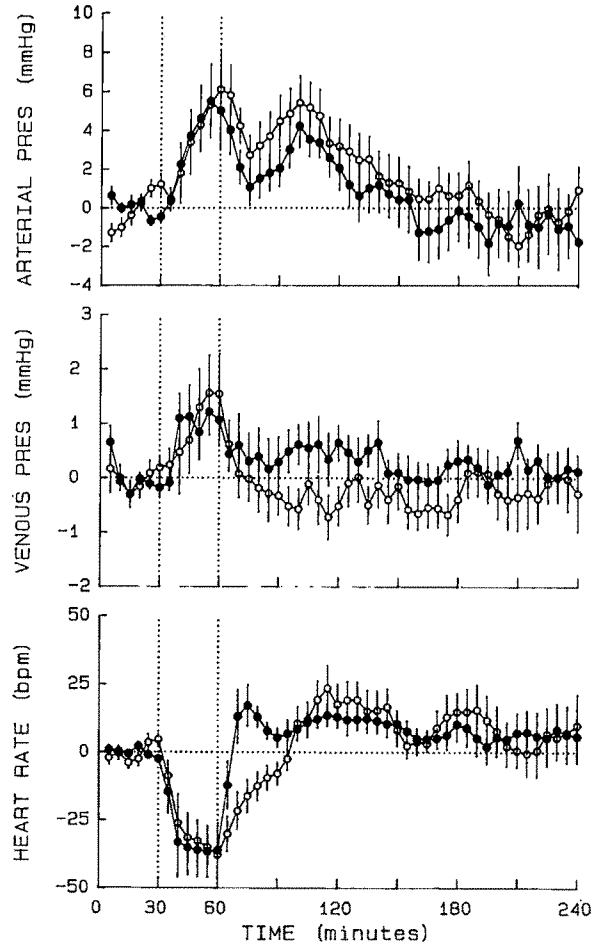


Fig. 2. Changes in fetal arterial pressure, venous pressure, and heart rate (mean ± SE) during control, hypoxic, and recovery periods in control *(open circles)* and arginine vasopressin–blocked *(closed circles)* fetuses. Data are expressed as change from mean control value during the first 30 minutes. *Vertical dotted lines* represent beginning and end of hypoxia.

Comment

Previous studies showed that moderate to severe hypoxia is a potent stimulus for release of arginine vasopressin into the fetal circulation and cerebrospinal fluid.[4, 11] In addition, intravenous infusions of exogenous arginine vasopressin into the ovine fetus increase arterial and venous pressures and decrease heart rate and blood volume.[4, 12-14] These cardiovascular responses to exogenous arginine vasopressin are all directionally consistent with those induced by fetal hypoxia. In addition, the increases in arterial and venous pressures and decreases in heart rate and blood volume during hypoxia coincide with the rise in plasma arginine vasopressin concentration.[4] Thus it was reasonable to hypothesize that, in the fetus, arginine vasopressin was responsible in part for the rise in arterial and venous pressures and decrease in heart rate and blood volume that occur in response to hypoxia. However, we found that blockade of the pressor effects of arginine vasopressin did not significantly alter the increases in arterial and venous pressures during hypoxia. Thus it appears that factors other than arginine vasopressin are responsible for the vascular pressure changes during acute hypoxia in the fetus. Our findings appear to contrast with a recent report of Perez et al.[22] who found that the hypertension during hypoxia in the sheep fetus was partially reversed when the same arginine vasopressin antagnoist as that used in our study was administered at the midpoint of the hypoxic period. However, although no statistical analyses were performed, it can be seen in the data of Perez et al.[22] that, even though the blocker lowered arterial pressure when administered during hypoxia, at no time was arterial pres-

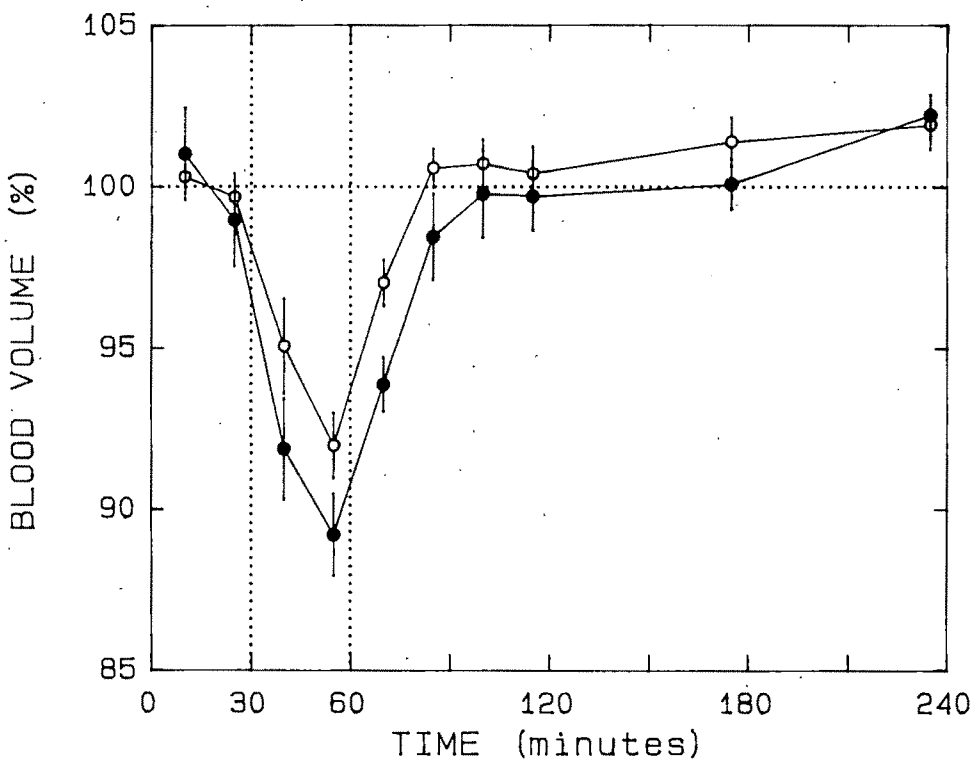


Fig. 3. Changes in blood volume (mean ± SE) during severe hypoxia in control *(open circles)* and arginine vasopressin–blocked *(closed circles)* fetuses. Data are expressed as percentage of mean control during the first 30 minutes. *Dotted vertical lines* represent beginning and end of hypoxia.

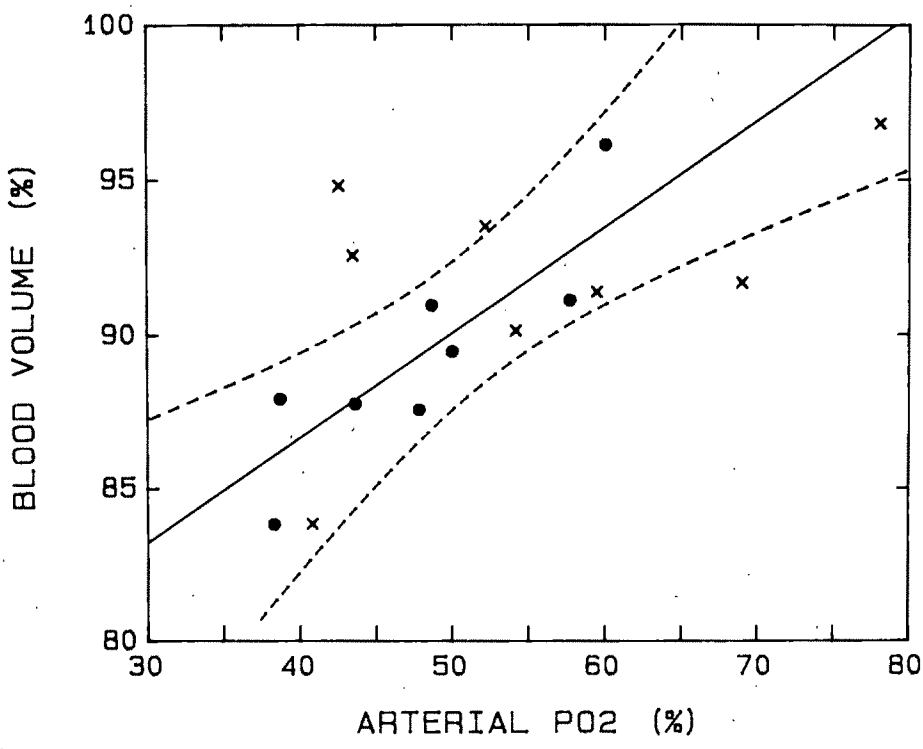


Fig. 4. Correlation between changes in arterial Po_2 and blood volume in control *(crosses)* and arginine vasopressin–blocked *(closed circles)* ovine fetuses at end of hypoxic period ($r = 0.653$, $p < 0.01$).

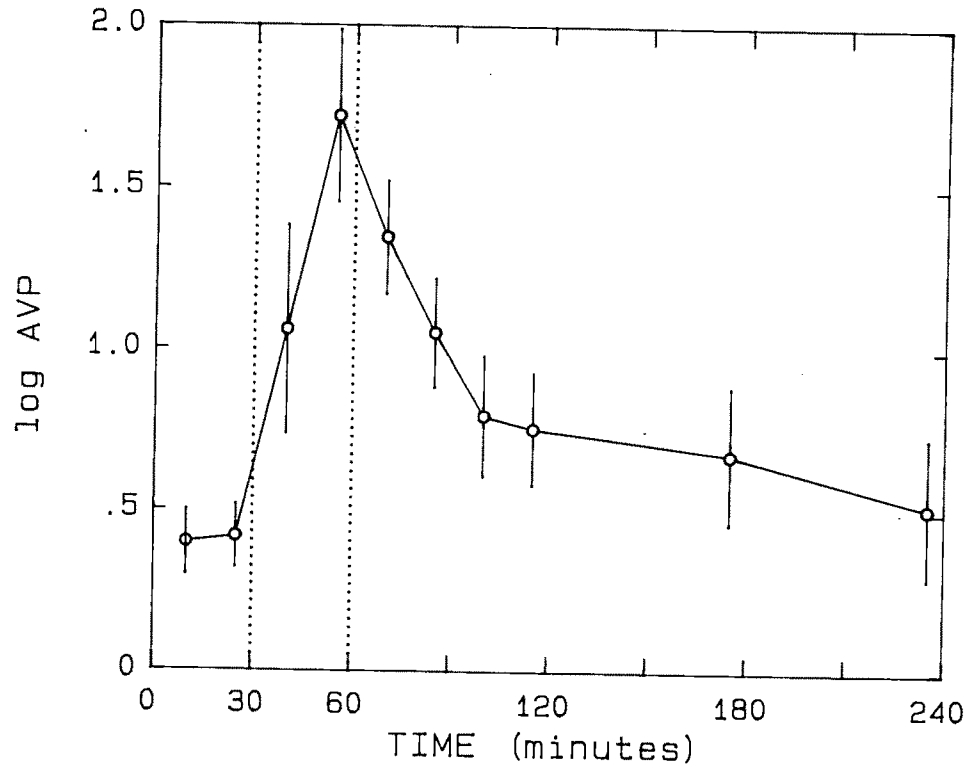


Fig. 5. Arterial arginine vasopressin (AVP) concentration in picograms per milliliter over time in control fetuses. *Vertical lines* represent beginning and end of hypoxia. Data presented are mean ± SE of log (base 10) transformed plasma arginine vasopressin concentrations.

sure in the blocked fetuses significantly different from that in the control fetuses. Thus the contribution of arginine vasopressin to the fetal arterial hypertension during acute hypoxemia is subtle and difficult to detect if it does indeed occur. Alternatively, it is possible that under the situation when the arginine vasopressin system is blocked during hypoxia, other pressor systems are activated to compensate for the lack of arginine vasopressin effects in the restoration of cardiovascular function to normal.

In addition to the arterial hypertension, an important component of the fetal cardiovascular response to acute severe hypoxia is venous hypertension. This study confirms our previous observation[10] of an elevation in venous pressure during hypoxia. Furthermore, this study shows that even if an arginine vasopressin blocker were present before the onset of hypoxia, the venous pressure response to hypoxia was not altered. Thus arginine vasopressin does not appear to be a primary mediator of the hypoxia-induced venous hypertension in the late gestation ovine fetus, even though exogenous arginine vasopressin at high doses elevates fetal venous pressure.[12]

We and others[4, 12-14] have shown that infusion of arginine vasopressin into the ovine fetus results in bradycardia. We speculated that the decrease in heart rate during hypoxia could be partially mediated by arginine vasopressin. However, because the decrease in heart rate during hypoxia was similar in both the control and arginine vasopressin–blocked fetuses, it appears that arginine vasopressin does not mediate the fetal heart rate changes during hypoxia. Subsequent to hypoxia we observed a tachycardia at the beginning of the recovery period in fetuses treated with the antagonist, whereas heart rate remained suppressed in control fetuses at this time. Inasmuch as the arterial and venous pressure responses at this time were similar between the two groups, these findings suggest that fetal heart rate in the immediate posthypoxic period may be modulated by a direct action of arginine vasopressin on heart rate, and this action is sensitive to blockade by the pressor antagonist. Alternatively, arginine vasopressin may alter fetal heart rate indirectly through interaction with other endocrine factors such as the catecholamines. Conclusions similar to ours were obtained from the study by Perez et al.[22] in which the fall in fetal heart rate during hypoxia was partially reversed by the arginine vasopressin antagonist. This conclusion is further supported by the present observation that the return of heart rate to basal levels in the control fetuses paralleled the return of plasma arginine vasopressin to basal concentrations.

Hypoxia induced a decrease in fetal blood volume that most likely results from an increase in capillary pressure.[10] We have previously demonstrated that the catecholamines may mediate this fall in blood volume only when the degree of hypoxia is mild.[10] With moderate to severe hypoxia (fall in Pa_{O_2} of >6 mm Hg), the blood volume changes can no longer be accounted for by the catecholamines, because combined α-adrenergic plus β-adrenergic receptor blockade does not alter the blood volume decrease during severe hypoxia.[10] It has been suggested that acidosis may contribute to the decrease in blood volume during hypoxia,[23] but the fall in blood volume precedes the decrease in blood pH.[10] Because infusion of arginine vasopressin into the ovine fetus decreases blood volume[12] and plasma arginine vasopressin concentrations increase during hypoxia when fetal Pa_{O_2} falls below 17 mm Hg,[15, 16] it was reasonable to speculate that the fall in fetal blood volume during severe hypoxia may be attributable to the rise in plasma arginine vasopressin concentrations. However, in the present study the fall in fetal blood volume was similar between the control and arginine vasopressin antagonist—treated groups, thus eliminating the possibility that arginine vasopressin might mediate this change in blood volume. In support of this finding was the fact that the increase in venous pressure in these fetuses was not altered by treatment with arginine vasopressin antagonist. In other words, with similar increases in venous and arterial pressures, it is not surprising to find similar changes in blood volume because the changes in capillary pressure are most likely similar.

In summary, circulating arginine vasopressin in the ovine fetus does not appear to mediate the arterial or venous hypertension or the hypovolemia induced during acute hypoxia. However, arginine vasopressin appears to mediate part of the posthypoxic bradycardia that occurs in these fetuses.

REFERENCES

1. Cohn HE, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. Am J Obstet Gynecol 1974;120:817-24.
2. Iwamoto HS, Rudolph AM, Mirkin BL, Keil LC. Circulating and humoral responses of sympathectomized fetal sheep to hypoxemia. Am J Physiol 1983;245:H767-72.
3. Lewis AB, Wolf WJ, Sischo W. Cardiovascular and catecholamine responses to successive episodes of hypoxemia in the fetus. Biol Neonate 1984;45:105-11.
4. Rurak DW. Plasma vasopressin levels during hypoxemia and the cardiovascular effects of exogenous vasopressin in foetal and adult sheep. J Physiol 1978;277:341-57.
5. Jones CT, Roebuck MM, Wallar DW, Johnston BM. The role of the adrenal medulla and peripheral sympathetic nerves in the physiological responses of the fetal sheep to hypoxia. J Dev Physiol 1988;10:17-36.
6. Jones CT, Robinson RO. Plasma catecholamines in fetal and adult sheep. J Physiol 1975;248:15-33.
7. Stegner H, Leake RD, Palmer SM, Oakes G, Fisher DA. The effect of hypoxia on neurohypophyseal hormone release in fetal and maternal sheep. Pediatr Res 1984; 18:188-91.
8. Jones CT, Ritchie JWK, Walker D. The effects of hypoxia on glucose turnover in the fetal sheep. J Dev Physiol 1983;5:223-35.
9. Cohen WR, Piasecki GJ, Cohn HE, Young JB, Jackson BT. Adrenal secretion of catecholamines during hypoxemia in fetal lamb. Am J Physiol 1982;243:R520-5.
10. Brace RA, Cheung CY. Role of catecholamines in mediating fetal blood volume decrease during acute hypoxia. Am J Physiol 1987;253:H927-32.
11. Stark RI, Daniel SS, Husain MK, Tropper PJ, James LS. Cerebrospinal fluid and plasma vasopressin in the fetal lamb: basal concentration and effect of hypoxia. Endocrinology 1985;116:65-72.
12. Tomita H, Brace RA, Cheung CY, Longo LD. Vasopressin dose-response effects on fetal vascular pressures, heart rate, and blood volume. Am J Physiol 1985;249:H974-80.
13. Gest AL, Moise AA, Hansen TN, Kaplan S. Effects of arginine vasopressin on hemodynamics and lung fluid balance in lambs. Am J Physiol 1989;256:H641-7.
14. Iwamoto HS, Rudolph AM, Keil LC, Heymann MA. Hemodynamic responses of sheep fetus to vasopressin infusion. Circ Res 1979;44:430-6.
15. Devane GW, Naden RP, Porter JC, Rosenfeld CR. Mechanism of arginine vasopressin release in sheep fetus. Pediatr Res 1982;16:504-7.
16. Stark RI, Wardlaw SL, Daniel SS, et al. Vasopressin secretion induced by hypoxia in sheep: developmental changes and relationship to β-endorphin release. Am J Obstet Gynecol 1982;143:204-5.
17. Brace RA. Thoracic duct lymph flow and its measurement in chronically catheterized sheep fetus. Am J Physiol 1989;256:H16-20.
18. Gleed RD, Poore ER, Figueroa JP, Nathanielsz PW. Modification of maternal and fetal oxygenation with the use of trancheal gas infusion. Am J Obstet Gynecol 1986;155:429-35.
19. Brace RA. Blood volume and its measurement in the chronically catheterized sheep fetus. Am J Physiol 1983; 244:H487-94.
20. Brace RA. Blood volume in the fetus and methods for its measurement. In: Nathanielsz PW, ed. Animal models in fetal medicine. New York: Perinatology Press, 1984:19-36.
21. Miner LK, Cheung CY, Brace RA. Autonomic modulation of fetal atrial natriuretic factor and urine flow responses to vascular volume expansion. Am J Obstet Gynecol 1988;159:1341-6.
22. Perez R, Espinoza M, Riquelme R, Parer JT, Llanos AJ. Arginine vasopressin mediates cardiovascular responses to hypoxemia in fetal sheep. Am J Physiol 1989;256: R1011-8.
23. Widness JA, Teramo KA, Clemons GK, et al. Temporal response of immunoreactive erythropoietin to acute hypoxemia in fetal sheep. Pediatr Res 1986;20:15-9.

Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions

III. Cytotoxic potential in vitro and characterization of antigen(s)

William H. Kutteh, MD, PhD,[a] Vicki V. Baker, MD,[a] and George J. Doellgast, PhD[b]
Birmingham, Alabama, and Winston-Salem, North Carolina

Cyst and ascites fluids from patients with ovarian epithelial neoplasms contain immunoglobulins with antitumor activity. Autologous antibodies bound to the cellular membrane fragments obtained from human ovarian neoplastic effusions react with cell-surface antigens on different human ovarian cell lines, surgical specimens of human ovarian adenocarcinoma, and human ovarian tumors grown in athymic Balb/c mice. The antibodies do not react with tissue preparations from normal human ovaries, other nonovarian normal or neoplastic tissues, and nonovarian human cell lines. These studies indicate that these antibodies are capable of complement-mediated lysis of human ovarian tumor cell lines in vitro. Preliminary characterization of the autologous ovarian tumor-associated antigen(s) indicates that it may be composed of three large-molecular-weight proteins of 182,000, 164,000, and 122,000 d. (AM J OBSTET GYNECOL 1990;163:1301-6).

Key words: Ovarian cancer, tumor-associated antigens, tumor immunology, autologous antibodies

Ovarian carcinoma is the fifth leading cause of cancer death in women and the leading cause of death from gynecologic malignancies. More than 12,000 women die annually from this type of cancer.[1] Despite extensive use of operation, radiotherapy, and chemotherapy, the age-adjusted cancer death rate for cancer of the ovary has demonstrated a gradual increase from four deaths per 100,000 female population in 1930 to the present rate of 10 deaths per 100,000 female population.[1] The deep location of the organ and the lack of early symptoms make the diagnosis of ovarian cancer difficult; two thirds of the cases are not diagnosed until regional or distant metastases have occurred.[2] The most effective therapeutic courses against cancer of the ovary can provide only palliation with uncertain prolongation of life.

The most effective means of increasing survival rate is through early diagnosis of patients with ovarian cancer; thus many research efforts are currently under way to devise such detection methods. The majority of these efforts focus on the identification of tumor-associated or tumor-specific antigens as demonstrated by Gall et al.[3] Cells undergo changes that often result in the expression of new or altered cell products and cell-surface components as a consequence of transformation to the neoplastic state. Current evidence suggests that several classes of tumor-associated substances exist: those that are unique to a given tumor, those that are common to all tumors of a given histopathologic type, those that are associated with a given organ or tissue, those that may represent embryonic or transplantation-like antigens, as well as those that are ubiquitous in normal allogenic and xenogeneic cells.[4, 5]

The autologous antibodies isolated from human ovarian membrane fragments in this laboratory have a high degree of specificity for ovarian tumors but do not bind to normal ovarian or nonovarian tissues, or to nonovarian neoplastic tissues. These antibodies differ significantly from those described by other investigators who have produced heterologous polyclonal and monoclonal antibodies to ovarian tumor antigens such as OTA,[3] OVC,[6] IOTA,[7] OvTAA,[8] OCA,[9] OCAA and others,[10] NB-70K,[11] and OC 125.[12] This laboratory has demonstrated that patients with ovarian neoplasms are capable of initiating a specific immune response characterized by antibodies that recognize autologous ovarian tumor-associated antigen(s).[13-15] In this study, human ovarian tumor cell lines have been used as a source for the characterization of cell-surface antigens

From the Department of Obstetrics and Gynecology, University of Alabama Medical Center,[a] and the Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University.[b]
Supported by grant IN-66Y from the American Cancer Society and a Mead Johnson—American College of Obstetricians and Gynecologists Fellowship Award.
Received for publication November 13, 1989; accepted May 1, 1990.
Reprint requests: William H. Kutteh, MD, PhD, Department of Obstetrics and Gynecology, J6.114, 5323 Harry Hines Blvd., University of Texas SW Medical Center, Dallas, TX 75235-9032.
6/1/22089

Table I. Membrane-bound immunoglobulin preparations

Sample	*Source*	*Pathologic diagnosis*	*Initial volume (ml)*	*IgG (ng/ml*)*
T.E.B.	Ovary	Adenocarcinoma	4200	850
J.B.S.	Ovary	Serous cystadenocarcinoma	8000	773
E.M.D.	Ovary	Papillary adenocarcinoma	200	500
V.I.F.	Ovary	Serous cystadenocarcinoma	250	400
W.M.J.	Colon	Crohn's disease	1500	600

*Amounts of membrane-bound IgG were determined by enzyme-linked immunoassay as described previously.[14] (Table modified from Kutteh WH, Welander CE, Doellgast GJ. JNCI 1986;76:797-804.)

reactive with membrane-eluted antibodies. These studies extend our previous reports by demonstrating that autologous antibodies eluted from membrane fragments of human ovarian neoplasms are capable of cytotoxicity against human ovarian cancer cell lines in vitro.

Material and methods

Preparation of membrane eluted antibodies. The detailed methods for preparation, purification, and specificity testing of ovarian membrane-eluted antibodies have been described.[14, 15]

Tissues and cells. Human use approval was granted through the Institutional Review Board for the collection and use of human tissue. Effusions from human ovarian epithelial neoplasms (T.E.B., J.B.S., E.M.D., and V.I.F.) and pleural fluid from a patient with Crohn's disease (W.M.J.) were obtained from the Pathology Services at the Bowman Gray School of Medicine (Table I). Human cell line No. 2774, derived from an ovarian serous cystadenocarcinoma, was provided by Phillip DiSaia, MD (University of California, Irvine, Orange, Calif.). Cell line No. 7315, also derived from an ovarian serous cystadenocarcinoma, was established at Bowman Gray School of Medicine.[14] Normal human tissues were obtained from the Tissue Procurement Service, Comprehensive Cancer Center (University of Alabama at Birmingham, Birmingham, Ala.). Human cell lines were maintained in culture in RPMI 1640 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum, penicillin (0.1 mg/ml), streptomycin (100 U/ml), and 2.0 mmol/L L-glutamine (Gibco) at 37° C in an atmosphere of 95% air and 5% oxygen. Peripheral blood lymphocytes used as control cells in the cytotoxicity assay (described below) were prepared from whole blood.[16]

Immunofluorescence. Two preparations of autologous ovarian membrane-eluted antibodies (T.E.B. and J.B.S.) were tested against a panel of human tumor cell lines, fresh human tumor tissue, and fresh normal human tissue by indirect immunofluorescence. Names of each cell line and tissue tested have been previously listed.[14] Adherent cells were removed from tissue culture flasks with 0.25% trypsin for 5 minutes, washed three times in phosphate-buffered saline solution that contained 1% bovine serum albumin and 0.03% sodium azide, and adjusted to a concentration of 1×10^7 cells/ml. Equal volumes of membrane-eluted antibodies, immunoglobulin G (IgG) from normal serum, or buffered saline solution were incubated with washed cells for 45 minutes at 22° C. Cells were washed twice and then incubated with the fluorescein-labeled antihuman IgG, IgM, and IgA (heavy-chain-specific) (Calbiochem-Behring, Davis, Calif.). After 45 minutes at 22° C the cells were washed twice, resuspended in buffer, mounted on a slide, and examined immediately. Tissue sections embedded in 7.5% buffered gelatin were frozen, cut, mounted, and then stained by means of similar techniques. Cells and tissue sections were visualized by means of an Olympus BH-2 microscope (Olympus Optical Co., Ltd., Tokyo) with epiillumination.

Immunoglobulins. Normal human IgG was used as one control source of antibody for immunofluorescence and in the cellular cytotoxicity assay. The methods used for the preparation of IgG from the sera of normal individuals have been described previously.[16]

Cytotoxicity assay. To determine whether purified autologous antibodies from patients with ovarian cancer were capable of cellular killing against human ovarian tumor cells in vitro, we used a complement-mediated cellular cytotoxicity assay.[17] Briefly, single-cell suspensions of ovarian tumor cells or control cells were prepared as described above and washed three times. Cells (5×10^4) were incubated with serial twofold dilutions of membrane-eluted antibodies or control immunoglobulin (10 μl) for 30 minutes at 37° C in 96-well U-bottom microplates (Costar, Cambridge, Mass.). Guinea pig serum was added as a source of complement (Pel-Freeze Biologicals, Rogers, Ark.) (10 μl) and cells were further incubated for 30 minutes at 37° C. The reaction was stopped with cold 0.015 mol/L ethylenediaminetetraacetic acid (Sigma, St. Louis) and then 10 μl of 0.4% trypan blue (Gibco) was added to each well to evaluate viability by the dye exclusion assay. The percent of cells taking up dye per total number of cells present was determined by light microscopy and is reported as percent killing. Each dilution was performed in triplicate with mean values reported. Similar results were obtained with ovarian tumor cells when single cell

Table II. Immunofluorescence specificity of autologous membrane antibodies

Source of cells	*Type of cells*	*No. positive/No. tested*
Human tumor cell lines	Ovarian adenocarcinoma	8/8
	Colon adenocarcinoma	0/6*
	Uterine carcinoma	0/4
	Breast adenocarcinoma	0/2
Neoplastic human tissues	Ovarian adenocarcinoma	8/8
	Colon adenocarcinoma	0/4*
	Uterine carcinoma	0/4*
Normal human tissues	Ovary	0/8
	Colon	0/4
	Uterus	0/4
	Fallopian tube	0/4
	Lung	0/2

*Trace immunofluorescence was observed in one colon adenocarcinoma cell line, and one sample each of neoplastic tissue obtained from a colon adenocarcinoma and a uterine carcinoma. (Table modified and updated from Kutteh WH, Welander CE, Homesley HD, Doellgast GJ. AM J OBSTET GYNECOL 1985;153:124-9.)

suspensions were obtained by either mechanical disruption or treatment with 1 mmol/L ethylenediaminetetraacetic acid.

Characterization of tumor-associated antigen(s). Cells from the human serous cystadenocarcinoma line No. 2774 (or No. 7315) were surface labeled with iodine 125–sodium by the lactoperoxidase method, cells were washed, and then membrane antigens solubilized with Triton X-100 (United States Biochemical Corp., Cleveland). Labeled antigens were precipitated with autologous ovarian membrane-eluted antibodies (T.E.B., J.B.S., E.M.D., or V.I.F.) or controls (W.M.J., normal IgG, or phosphate-buffered saline solution) and immune complexes were precipitated with goat antihuman IgG. The precipitates were washed to remove excess radioactivity, reduced with β-mercaptoethanol, and electrophoresed on 7.5% reducing polyacrylamide gels. Gels were sliced into 1 mm sections, slices were counted for radioactivity, and counts per minute per slice were plotted based on relative mobility of molecular weight standards.[18]

Results

Specificity of membrane-eluted immunoglobulins. To determine whether these membrane-bound immunoglobulins demonstrated any activity against ovarian tumors, immunoglobulins were eluted from membrane fragments, and their binding activity to various normal and neoplastic tissues was investigated. The autologous membrane-eluted antibodies reacted strongly

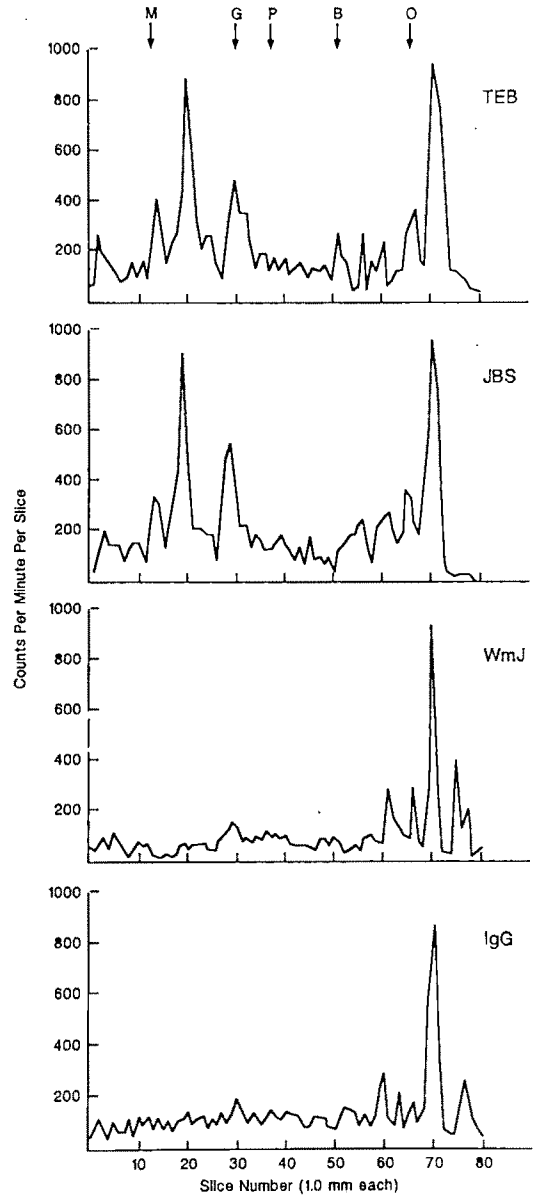


Fig. 1. Molecular weight characterization of autologous ovarian tumor-associated antigen. Human ovarian cystadenocarcinoma cell line No. 2774 was surface labeled with ^{125}I-sodium; cells were washed then solubilized with Triton X-100. Solubilized material was precipitated with autologous ovarian membrane-eluted antibody (T.E.B. or J.B.S.) or control immunoglobulin (W.M.J. or normal IgG); precipitates were washed and electrophoresed on 7.5% reducing polyacrylamide gels. Graphs depict counts per minute per 1 mm slice compared with relative mobility of molecular weight standards (*M*, myosin, 205,000 d; *G*, β-galactosidase, 116,000 d; *P*, phosphorylase B, 97,500 d; *B*, bovine serum albumin, 67,000 d; *O*, ovalbumin, 45,000 d). Dye front and free ^{125}I migrated with slice number 70. The figure represents one of four similar experiments.

with all human ovarian tumor cell lines and all surgical samples of human ovarian adenocarcinoma tested (Table II). The membrane-eluted antibodies either did not react (or reacted only weakly) with human tumor

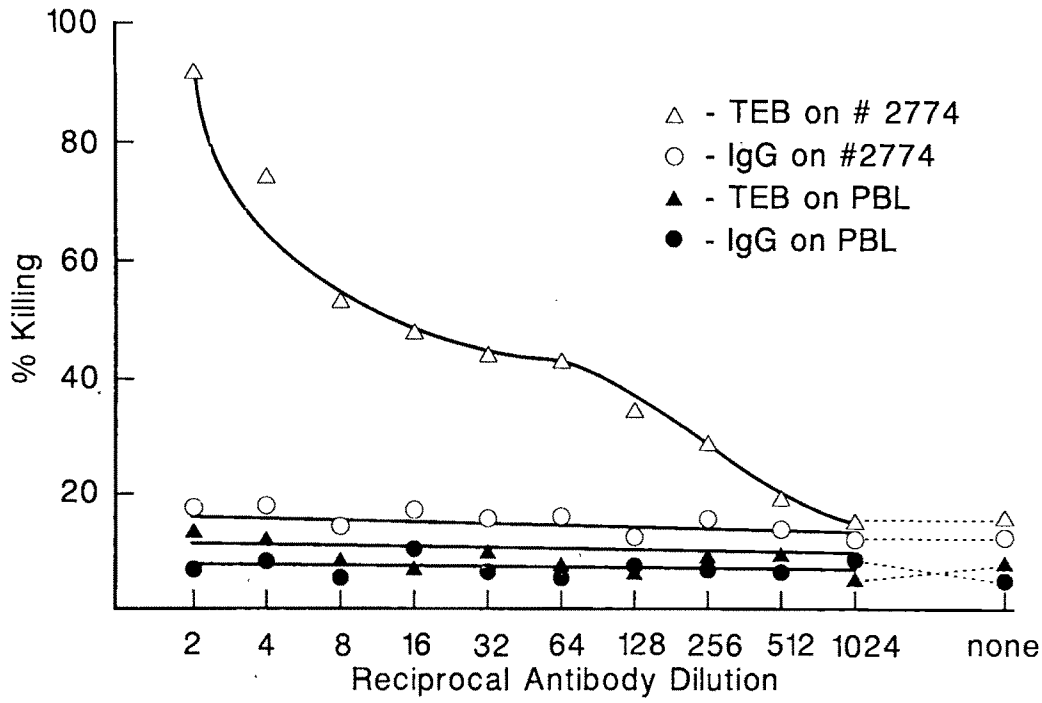


Fig. 2. Cytotoxic activity of autologous antibodies. Human ovarian tumor cell line No. 2774 or peripheral blood lymphocytes *(PBL)* (5×10^4) were washed and incubated with serial twofold dilutions of acid-eluted membrane antibodies (T.E.B.) or normal human IgG (10 μl) for 30 minutes at 37° C in microtiter plates. Guinea pig complement (10 μl) was added and cells were further incubated for 30 minutes at 37° C. The reaction was stopped with cold 0.015 mol/L ethylenediaminetetraacetic acid and 10 μl of 0.4% trypan blue was added to each well. Percent of cells taking up dye per total number of cells present was determined by light microscopy. Plotted points represent mean value of triplicates. *Open triangles*, T.E.B. on No. 2774; *open circles*, IgG on No. 2774; *solid triangles*, T.E.B. on *PBL*; *closed circles*, IgG on *PBL*.

cell lines and neoplastic tissue from colon, uterus, and breast. No reactivity was detected against normal human ovary, colon, uterus, lung, fallopian tube, or small intestine.

Characterization of autologous ovarian tumor-associated antigen (Fig. 1). Our results indicate that a tumor-associated antigen(s) is present on human ovarian tumor cells that is recognized by several of our autologous membrane-eluted antibody preparations. Antibody preparations from the fluids of four patients (T.E.B., J.B.S., E.M.D., and V.I.F.) precipitated the major bands corresponding to molecular weights of 182,000, 164,000, and 122,000 d. These bands were absent in control precipitates (buffered saline solution, W.M.J., normal IgG). These major bands may represent subunits of one antigen or three separate cell-surface tumor-associated antigens. Indeed, analysis of areas under the peaks of radioactivity demonstrates a consistent ratio of 1 : 4 : 2 (corresponding to molecular weight bands of 182,000 d : 164,000 d : 122,000 d).

Cytotoxic activity of autologous ovarian tumor-associated antigen (Fig. 2). Ovarian membrane-eluted antibodies were tested for cytotoxic activity against ovarian tumor cell lines that have positive reactivity by immunofluorescence.[14] Fig. 2 is representative of four experiments and demonstrates greater than 80% killing of cell line No. 2774 when incubated with two of our autologous antibody preparations (T.E.B. and J.B.S.). This cytotoxic activity decreased to background (20%) with dilution of antibody. No specific killing above background was demonstrated with control antibody on ovarian tumor cells or with specific antibody on unrelated cells. Similar results were obtained with cells from the serous cystadenocarcinoma line No. 7315.

Comment

The detection of ovarian cancer at an earlier stage of development might be improved if these isolated autologous antibodies prove to be as specific in vivo as they appear to be for the ovarian cancer cell in vitro. Theoretically, tumor-associated antigens could be recognized by the host and serve as a stimulus for an immune response in vivo. Numerous investigators have evidence for specific cell-mediated immunity and several laboratories have described the presence of specific antibody bound to ovarian tumor cells, suggesting that the patient has the capability of initiating an immune response against the tumor.[13, 19, 20]

Hellstrom et al.[21] describe the in vitro destruction of ovarian tumor cells by patients' lymphocytes in a colony inhibition assay. Chen et al.[22] used a leukocyte migration assay to test for hypersensitivity to tumor antigens. Levin et al.[23] describe cell-mediated immunity in the form of the blastogenic response of lymphocytes from ovarian cancer patients to crude cell extracts of ovarian tumor cells. The response of patients in remission was greater than that of normal controls, relapse patients, or patients with other cancers. These studies provide inconclusive data concerning the possibility of the in vivo response of the host to the developing tumor. However, if the tumor cells are coated with antibody in vivo, complement fixation and cell lysis should occur. Indeed, the presence of bound C3 on ovarian carcinoma cells from peritoneal effusions has been reported,[20] an indication that the first few components in the cascade are activated; however, the cells remain viable. The antibody may have its Fc fragment partially degraded by enzymes present in the fluid surrounding the tumor, there may be complement deficiencies present, or certain blocking factors may inhibit cytolysis.[24] In our laboratory two of the autologous antibody preparations were capable of complement-mediated lysis in vitro against human ovarian carcinoma cell lines but not against peripheral blood mononuclear cells or unrelated cell lines. It is possible that fresh ovarian tumor cells are resistant to complement-mediated cellular killing, whereas tissue culture adopted cells have lost this property. However, we are not aware of any previous reports in the literature in which this has occurred. We have been unable to test the autologous antibodies for the ability to mediate cellular cytotoxicity against fresh ovarian neoplastic tissue because of the spontaneous cell death that occurs on transfer of freshly dispersed tumor cells into culture media.

Several research groups have identified antigens associated with epithelial ovarian tumors and have been successful in producing antibodies in experimental laboratory animals. Most groups have reported that the ovarian tumor-associated antigens are high molecular weight. The antigens have been characterized as β-globulins; they are distinct from carcinoembryonic antigen, α-fetoprotein, ferritin, and pregnancy-associated antigens.[8, 25] The antigens are glycosylated and appear to be stable to perchloric acid extraction.[6, 9, 25] Our results suggest that autologous ovarian tumor-associated antigen may exist as a trimer with an apparent molecular weight of 468,000 d composed of three subunits of 182,000, 164,000, and 122,000 d. Alternatively, these molecular weights may represent three separate antigens.

Our experiments indicate that complete functional immunoglobulins with biologically active Fab and Fc regions were produced in vivo by patients with ovarian adenocarcinoma and that these autologous antibodies maintained cytotoxic activity against ovarian neoplastic cells in vitro. The observations that tumors continue to grow in the presence of a specific host immune response suggest the existence of mechanism(s) that enable tumors to escape immune destruction. Factors that suppress the blastogenic response of normal human lymphocytes have been detected in ascites[26] and serum[27] of patients with ovarian cancer. It has also been suggested that shedding of antigens may provide protection for the tumor by competing for the effector process of the immune system.[28, 29] In a recent report, IgG dissociated from immune complexes in ovarian ascitic fluid was able to detect significantly elevated levels of tumor-associated antigens in sera of patients with ovarian cancer compared with controls.[30] Additional work is needed to determine whether these antigens dissociated from circulating immune complexes are related to autologous ovarian tumor-associated antigen. The roles that autologous ovarian tumor-associated antigen and autologous antibodies to autologous ovarian tumor-associated antigen play in the host response to ovarian malignancy and their manipulation for detection, monitoring, and possible therapy are the goals of current investigation.

REFERENCES

1. Silverberg E, Boring CC, Squires TS. Cancer statistics, 1990. CA 1990;40:9-26.
2. Barber HRK, Dorsett B. The immune system in gynecologic malignancies. In: Gleicher N, ed. Reproductive immunology. New York: Alan R. Liss 1989;357-69.
3. Gall SA, Walling J, Pearl J. Demonstration of tumor-associated antigens in human gynecologic malignancies. Am J Obstet Gynecol 1973;115:387-93.
4. Benson MD, Lurain JR, Newton M. Ovarian tumor antigens. J Reprod Med 1983;28:17-23.
5. Bast RC, Knapp RC. The immunobiology of ovarian cancer. In: Griffiths CT, Fuller AF, eds. Gynecologic oncology. Boston: Martinus Nijhoff, 1983:187-226.
6. Imamura N, Takahashi T, Lloyd KO, Lewis JL, Old LJ. Analysis of human ovarian tumor antigens using heterologous antisera: detection of new antigenic systems. Int J Cancer 1978;16:570-7.
7. Stolbach LL, Cantantarow WD, Gronin WJ, et al. Dissociation and characterization of ovarian cancer antigen (Ag) and antibody (Aby) from immune complexes. Proc Am Assoc Cancer Res 1980;21:239.
8. Dawson JR, Kutteh WH, Whitesides DB, Gall SA. Identification of tumor-associated antigens and their purification from cyst fluids of ovarian epithelial neoplasms. Gynecol Oncol 1980;10:6-17.
9. Knauf S, Urbach GI. A study of ovarian cancer patients using a radioimmunoassay for human ovarian tumor-associated antigen OCA. Am J Obstet Gynecol 1980; 138:1222-3.
10. Bhattacharya M, Chatterjee SK, Barlow JJ, Fuji H. Monoclonal antibodies recognizing tumor-associated antigen of human ovarian mucinous cystadenocarcinomas. Cancer Res 1982;42:1650-54.
11. Bizzari JP, MacKillop WJ, Buick RN. Cellular specificity of NB70K, a putative human ovarian tumor antigen. Cancer Res 1983;43:864-7.

Mechanism of action of interleukin-1β in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells

Felice Petraglia, MD, Gian Carlo Garuti, MD, Biagina De Ramundo, MD, Stefano Angioni, MD, Andrea R. Genazzani, MD, and Louise M. Bilezikjian, PhD

Modena, Italy, and La Jolla, California

The present study evaluated the possible effect and mechanism of action of interleukin-1β in regulating the release of corticotropin-releasing factor and adrenocorticotropin hormone from human cultured placental cells. With the use of a primary monolayer culture of human placental cells at term, the addition of interleukin-1β increased the release of immunoreactive corticotropin-releasing factor with a dose- and time-dependent effect. The intracellular concentration of both cyclic adenosine monophosphate and cyclic guanosine monophosphate increased in the presence of interleukin-1β. The addition of indomethacin, a prostaglandin synthesis inhibitor, partially reversed the effect of interleukin-1β. The same doses of interleukin-1β stimulated the release of adrenocorticotropin hormone and this effect was partially reversed by the addition of a synthetic corticotropin-releasing factor antagonist or by indomethacin. This study showed that interleukin-1β increases the release of corticotropin-releasing factor and adrenocorticotropin hormone from cultured placental cells. This effect is associated with increased intracellular cyclic nucleotide concentrations and is in part reversed by a prostaglandin synthesis inhibitor. (AM J OBSTET GYNECOL 1990;163:1307-12.)

Key words: Interleukin-1β, corticotropin-releasing factor, adrenocorticotropin hormone, cyclic nucleotides, prostaglandins, placenta

The hormonal messengers of the immune system are referred to as cytokines. Among the cytokines, the interleukins are the leukocyte-derived peptides that play a role in mediating the immune response, activating hormonal sections, and influencing cell proliferation and differentiation.[1] In particular, the biologic effects of interleukin-1 have been largely investigated. The increased activity of the hypothalamus-pituitary-adrenal axis after the administration of interleukin-1 is one of the most interesting aspects of the immune-endocrine interaction.[2] The action of interleukin-1 is mediated by the release of hypothalamic corticotropin-releasing factor and seems to be involved in stress-induced responses.[2]

In view of the increased amniotic fluid interleukin-1 levels during labor[3] and its stimulatory effect on prostaglandin production,[4] a possible role of interleukin-1 in human parturition has been suggested. Recent studies[5-7] showed that bioactive interleukin-1 is present both in trophoblastic and decidual tissue, suggesting extra sources of this cytokine in pregnant women.

A possible role of placental corticotropin-releasing factor in human parturition has also been suggested. Indeed, placental contents and circulating levels of corticotropin-releasing factor increase during labor.[8] Moreover, stress-related factors (norepinephrine, vasopressin, glucocorticoids) increase corticotropin-releasing factor release and messenger ribonucleic acid levels in cultured placental cells.[9, 10] In agreement with its neuroendocrine effect, interleukin-1α increases the release of immunoreactive corticotropin-releasing factor from cultured placental cells.[9]

Because two distinct interleukin-1 subtypes have been identified by complementary deoxyribonucleic acid sequencing: interleukin-1α and interleukin-1β, active on the same cell membrane receptors,[1] in the present study we compared the effect of these two forms of interleukin-1 on immunoreactive corticotropin-releasing factor release from cultured human placental cells. Moreover, with the aim to elucidate the mechanism of action of interleukin-1β in a system of placental cells in culture, we measured: (1) the intracellular con-

From the Department of Obstetrics and Gynecology, University of Modena School of Medicine, and The Clayton Foundation Laboratories for Peptide Biology, Salk Institute.

Supported in part by the Consiglio Nazionale delle Ricerche (P.F. FATMA, SP.7.2.3) and by National Institutes of Health Grant No. DK26741.

Received for publication November 27, 1989; revised April 9, 1990; accepted May 11, 1990.

Reprint requests: Felice Petraglia, MD, Department of Obstetrics and Gynecology, University of Modena, School of Medicine, via del Pozzo 71, Modena, Italy 41100.

6/1/22420

centrations of cyclic adenosine monophosphate and cyclic guanosine monophosphate; (2) the release of immunoreactive corticotropin-releasing factor in the presence of indomethacin, a prostaglandin synthesis inhibitor; and (3) the release of immunoreactive adrenocorticotropin hormone in the presence of indomethacin or of a synthetic corticotropin-releasing factor antagonist.

Material and methods

Cell culture. Placental tissue was obtained from pregnant women ($n = 8$) undergoing elective cesarean section at term. Chunks of placental cotyledons were minced, rinsed, and dissected free from the connective tissue. Stirred in a water-jacketed flask (Wheaton Scientific, Millville, N.J.) at 300 rpm at 36° C, cells were enzymatically dispersed with 0.4% collagenase (Cooper Biochemicals Inc., Malvern, Pa.) and 3000 K units deoxyribonuclease type IV (Sigma Chemical Co., St. Louis) for 1 hour. A brief exposure to 0.4% trypsin (Sigma) (stirred for 10 minutes), was followed by a filtration on 42 μm filter.[11] The dissociated cells were washed twice in a mixture of HEPES dissociated buffer and culture medium Dulbecco's Eagle modified medium nutrient mixture F12 ham (Gibco, Painsley, U.K.) supplemented with Sato's cocktail and 10% fetal calf serum (Gibco).

Recombinant interleukin-1α and interleukin-1β (Roche, Basel, Switzerland), synthetic corticotropin-releasing factor antagonist (12-41) (Dr. J. Rivier, Salk Institute, La Jolla, Calif.) or indomethacin (Sigma) a cyclooxygenase inhibitor, were used in the experiments. Cultured cells were used 5 days after plating. At this time, after the present culture method, the percentage of cytotrophoblasts that had formed syncytiotrophoblasts was lower than 50%, thus allowing the presence of both cell populations in the culture system. Cultured cells were used 5 days after plating. On the day of the experiment, cells were washed twice with HEPES-buffered Krebs-Ringer bicarbonate solution and preincubated 1 hour with 1 ml of the same solution. Interleukin-1α or interleukin-1β, corticotropin-releasing factor antagonist, or indomethacin were dissolved in sterile distilled water and prepared as a 100-fold concentrate and added in small volumes to triplicate wells that contained 1 ml of 1% bovine serum albumin HEPES-buffered Krebs-Ringer bicarbonate solution, plus bacitracin (60 μl). Vehicle-treated wells (controls) were present in each experiment. Corticotropin-releasing factor antagonist or indomethacin was preincubated for 1 hour before the addition of interleukin-1β. The same experiment was repeated at least three times in cultures prepared from different subjects.

Corticotropin-releasing factor and adrenocorticotropin hormone assay. After 3 hours' incubation, conditioned media was removed and immunoreactive corticotropin-releasing factor and immunoreactive adrenocorticotropic hormone concentrations were measured by double-antibody radioimmunoassay. Synthetic rat corticotropin-releasing factor was used to prepare the standard curve and for iodination (chloramine-T method) to produce the tracer. Rabbit antirat corticotropin-releasing factor was used at the final dilution of 1:770,000. The same buffer as for adrenocorticotropin hormone radioimmunoassay was used. The details of the incubation procedures were previously described.[11] The assay sensitivity was 2 pg/tube. The interassay and intraassay coefficients of variation were 8% and 4%, respectively.

Porcine adrenocorticotropin hormone was used to prepare the standard curve and for iodination (chloramine-T method) to prepare the tracer. Ovine antihuman adrenocorticotropic hormone serum was used at a final dilution of 1:250,000. A 0.1% bovine serum albumin, 0.1% Triton X-100 saline phosphate buffer was used to dilute all reagents. The details of the incubation procedures were previously described.[11] The assay sensitivity was 2.0 pg/tube. The interassay and intraassay coefficients of variation were 8% and 4%, respectively.

Cyclic adenosine monophosphate and cyclic guanosine monophosphate assay. On removal of the conditioned medium, total intracellular cyclic nucleotides were extracted. Cells were immediately frozen on dry ice and 1 ml of 95% ethanol containing 0.1N hydrochloric acid was added. The cells were scraped and the solution was placed in glass borosilicate tubes. This procedure was repeated twice. The extracts were stored at −20° C overnight. Centrifugation at 2500 *g* at 4° C for 45 minutes allowed denatured proteins to precipitate, whereas the supernatants were dried in a speed-vacuum concentrator (Savant, Hicksville, N.J.). The final recovery of both cyclic nucleotides was 95%, as monitored with either cyclic nucleotides labeled with iodine 125 or a known amount of standard.[12] Redissolved in sodium acetate buffer, duplicate samples were submitted to specific double-antibody radioimmunoassays for cyclic adenosine monophosphate and cyclic guanosine monophosphate.

Kits were purchased by Biomedical Technologies Inc. (Stoughton, Mass.) to measure cyclic nucleotides on the basis of methods previously described.[13] The antisera did not recognize the other cyclic and noncyclic nucleotides. The assay sensitivity was 0.005 pmole/tube for both cyclic nucleotides measured. The interassay and intrassay coefficients of variation were 8% and 3%, respectively, for cyclic adenosine monophosphate, and

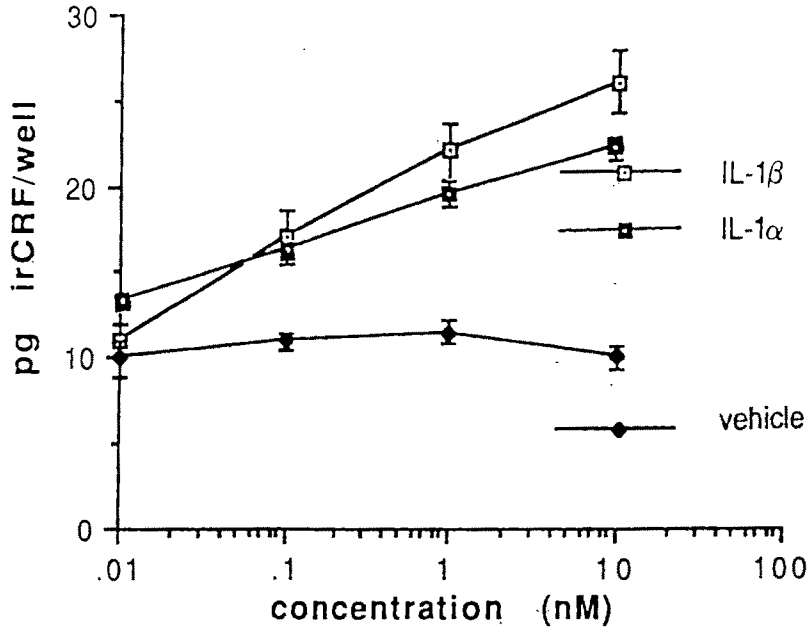


Fig. 1. Dose-dependent effect of interleukin-1 *(IL-1α)* or interleukin-1β *(IL-1β)* on immunoreactive corticotropin-releasing factor *(irCRF)* release from cultured placental cells. Each point represents mean ± SE.

9% and 4%, respectively, for cyclic guanosine monophosphate.

Results

Interleukin-1β significantly increased the release of immunoreactive corticotropin-releasing factor from cultured placental cells with a dose-dependent effect ($p < 0.01$ vs vehicle) (Fig. 1). The interleukin-1β–induced release of immunoreactive corticotropin-releasing factor was similar to that stimulated by equimolar doses of interleukin-1α (Fig. 1). Intracellular concentrations of cyclic adenosine monophosphate and cyclic guanosine monophosphate singificantly increased after the addition of interleukin-1β to the culture medium. The effect of interleukin-1β was time-dependent (the highest values were found after 30 minutes; data not shown) and dose-dependent ($p < 0.01$) (Fig. 2). Evaluated as maximum percentage increase over control values, the mean plus SD increase of cyclic guanosine monophosphate (550 + 75%) was significantly higher than that of cyclic adenosine monophosphate (185 + 45%) ($p < 0.01$).

The addition of indomethacin significantly decreased immunoreactive corticotropin-releasing factor release induced by interleukin-1β ($p < 0.01$) (Fig. 3).

Under the same experimental conditions, interleukin-1β significantly increased immunoreactive adrenocorticotropic hormone release (Fig. 4). The addition of a synthetic corticotropin-releasing factor antagonist or of indomethacin significantly decreased the interleukin-1β–induced release of immunoreactive adrenocorticotropic hormone ($p < 0.01$) (Fig. 4). When added together the corticotropin-releasing factor antagonist and indomethacin further decreased the release of immunoreactive adrenocorticotropin hormone induced by interleukin-1β ($p < 0.01$) but did not completely reverse the effect (data not shown).

Comment

The present study showed that similar to the systemic effect on hypothalamus-pituitary adrenal axis, interleukin-1β and interleukin-1α increase the release of immunoreactive corticotropin-releasing factor from cultured placental cells. This effect associated with the release of immunoreactive adrenocorticotropin hormone and both these actions of interleukin-1β are in part mediated through the activation of the prostaglandin pathway. Moreover, the increase of intracellular cyclic adenosine monophosphate and cyclic guanosine monophosphate concentrations during interleukin-1β incubation indicated that both cyclic nucleotide pathways are activated by this cytokine.

The effect of interleukin-1β on placental immunoreactive corticotropin-releasing factor release was dose dependent and its action was equipotent to that of interleukin-1α. The evidence that interleukin-1β increases immunoreactive adrenocorticotropin hormone release and that this effect is in part inhibited in the presence of a corticotropin-releasing factor antagonist indicates that interleukin-1β stimulates the local inter-

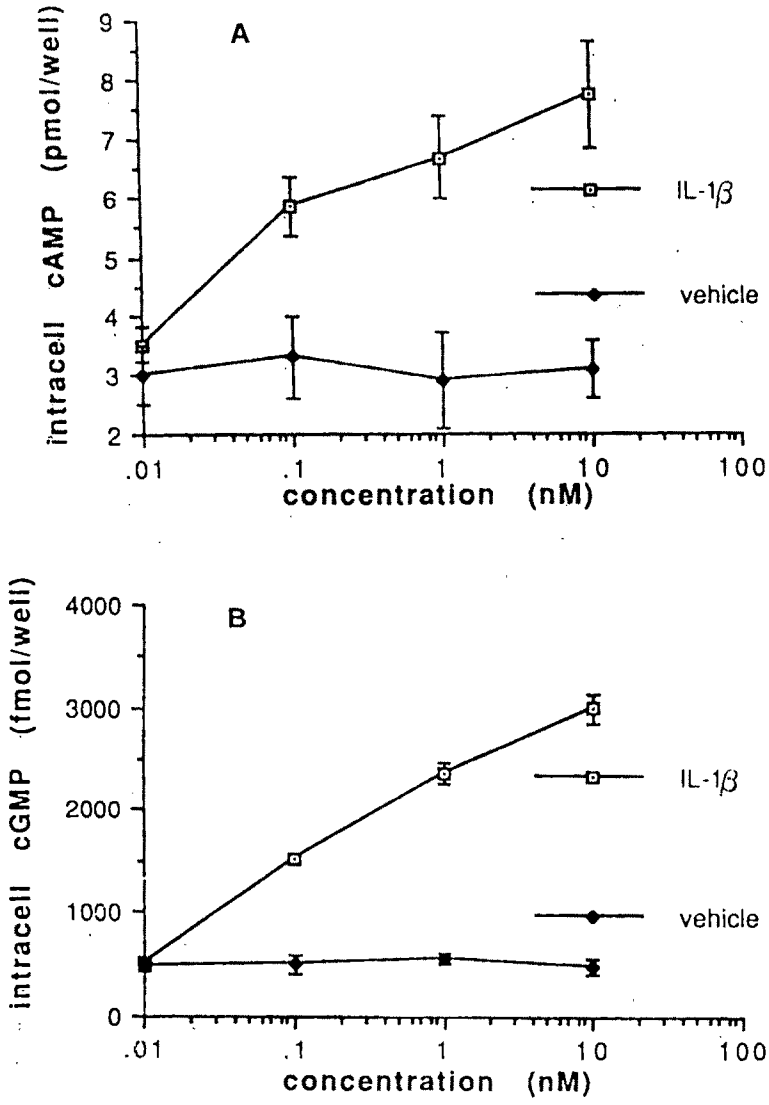


Fig. 2. Dose-dependent effect of interleukin-1β *(IL-1β)* on intracellular cyclic adenosine nucleotides *(cAMP) (panel A)* or on cyclic guanosine monophosphate *(cGMP) (panel B)*. Each point represents mean ± SE.

action between immunoreactive corticotropin-releasing factor and immunoreactive adrenocorticotropin hormone. Moreover, because indomethacin partially reverses the interleukin-1β–induced release of both immunoreactive corticotropin-releasing factor and immunoreactive adrenocorticotropin hormone, it may be suggested that interleukin-1β may modulate production of these placental hormones through prostaglandin release. This agrees with our previous finding that prostaglandins increase immunoreactive corticotropin-releasing factor and immunoreactive adrenocorticotropin hormone release from cultured placental cells.[11] The evidence that part of the interleukin-1β effect remains in the presence of indomethacin plus the corticotropin-releasing factor antagonist may be explained by the activity of other metabolites of the arachidonic acid. These findings obtained in vitro support the existence of paracrine or autocrine interactions between hormonal products in human placenta.

The increased intracellular cyclic adenosine monophosphate and cyclic guanosine monophosphate concentrations in the presence of interleukin-1β suggests a possible participation of these cyclic nucleotides in the hormonal effects of interleukin-1β in human placental cultured cells. In agreement with this hypothesis, the activation of the adenylate cyclase pathway was shown

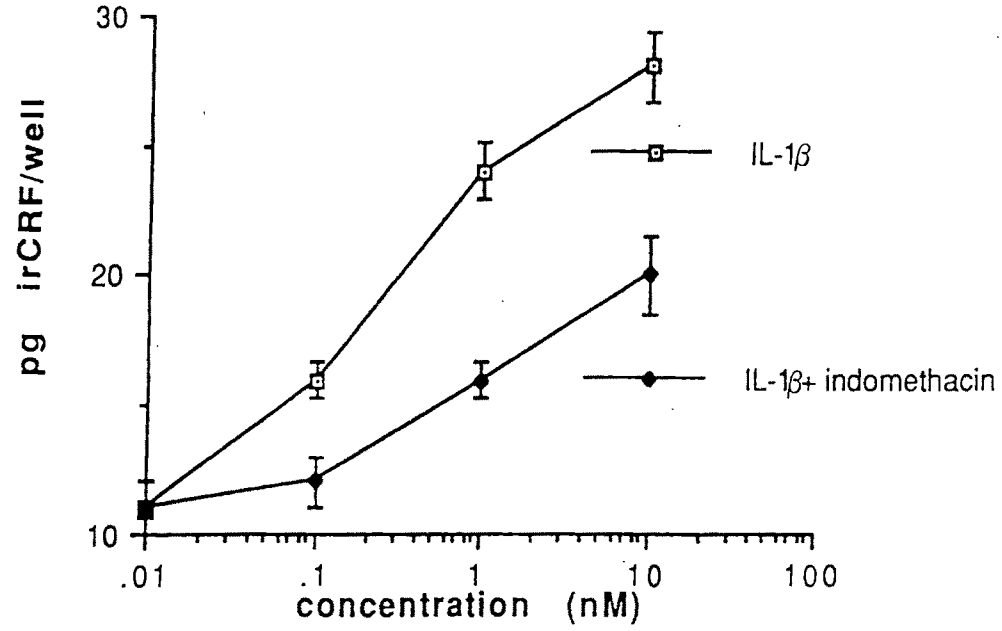


Fig. 3. Indomethacin (10 μmol) partially reverses effect of interleukin-1β *(IL-1β)* on immunoreactive corticotropin-releasing factor *(irCRF)* release from cultured placental cells. Each point represents mean ± SE.

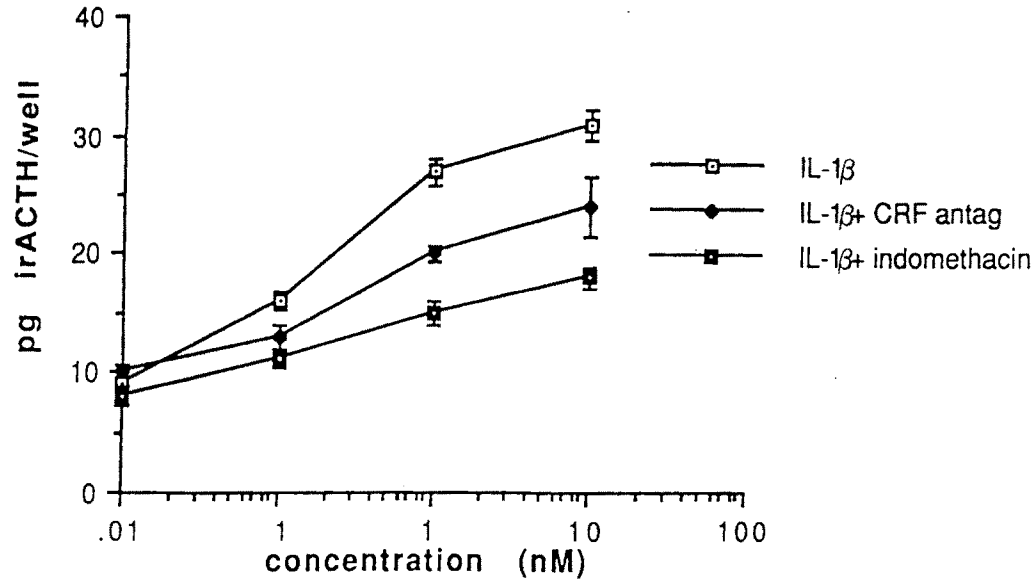


Fig. 4. Corticotropin-releasing factor *(CRF)* antagonist or of indomethacin partially reverses effect of interleukin-1β *(IL-1β)* on immunoreactive corticotropin-releasing factor *(irCRF)* release from cultured placental cells. Each point represents mean ± SE.

to stimulate the release of immunoreactive adrenocorticotropic hormone from cultured placental cells.[11]

The possible participation of interleukin-1 to the mechanisms of control of placental hormone production was recently supported by evidence that the addition of interleukin-1 to trophoblast cultures stimulated the release of human chorionic gonadotropin.[14]

The presence of interleukin-1 in trophoblast and decidua[5-7] supports a possible local source of the cytokines in modulation of placental hormone release. Hu-

man decidua also produces tumor necorsis factor-α, another cytokine, which shares several biologic properties with interleukin-1.[15] Therefore decidual or placental interleukin-1 may stimulate trophoblast cells to release corticotropin-releasing factor and it may be suggested to contribute to the increase of plasma corticotropin-releasing factor and adrenocorticotropin hormone levels during labor.[8] Moreover, the observation that both interleukin-1 and corticotropin-releasing hormone increase prostaglandin synthesis in cultured human decidual tissue[4, 16] further sustains the hypothesis that an immune-endocrine interaction may play a role in human parturition. This hypothesis has been mainly supported by the observations obtained in preterm labor.[3, 15, 16] However, the present and previous results were obtained in vitro and at and the present time it is speculative to hypothesize that this interaction occurs in vivo.

In conclusion, the present results show that interleukin-1β stimulates the corticotropin-releasing factor, adrenocorticotropin hormone axis in a system of cultured placental cells involving a prostaglandin pathway, thus suggesting the existence of an immune-hormonal interaction in human placenta.

REFERENCES

1. Dinarello CA. Biology of interleukin. FASEB J 1988; 2:108-15.
2. Bateman A, Singh A, Kral T, Solomon S. The immune-hypothalamic-pituitary-adrenal axis. Endocr Rev 1989; 10:92-111.
3. Romero R, Brody DT, Oyarzun E, et al. interleukin-1: a signal for the onset of parturition. AM J OBSTET GYNECOL 1989;160:1117-23.
4. Romero R, Durum S, Dinarello C, et al. Interleukin-1 stimulates prostaglandin biosynthesis by human amnion. Prostaglandins 1989;37:13-22.
5. Flynn A, Finke J, Hilfinker ML. Placental mononuclear phagocytes as a source of interleukin-1. Science 1982; 218:475-6.
6. Main EK, Strizki J, Schochet P. Placental production of immunoregulatory factors: trophoblast is a source of interleukin-1. Trophobl Res 1987;2:149-52.
7. Romero R, Wu JK, Brody DT, et al. Human decidua: a source of interleukin-1. Obstet Gynecol 1989;73:31-4.
8. Sasaki A, Shinkawa O, Margioris AN, et al. Immunoreactive corticotropin-releasing hormone in human plasma during pregnancy, labor and delivery. J Clin Endocrinol Metal 1987;64:224-9.
9. Petraglia F, Sutton S, Vale W. Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells. AM J OBSTET GYNECOL 1989;160:247-51.
10. Robinson BG, Emanuel RL, Frim DM, Majzoub JA. Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. Proc Natl Acad Sci USA 1988;85:5244-8.
11. Petraglia F, Sawchenko PE, Rivier J, Vale W. Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placental. Nature 1987;328: 717-9.
12. Bilezikjian LM, Vale W. Glucocorticoids inhibit corticotropin-releasing factor-induced production of adenosine 3',5'-monophosphate in cultured anterior pituitary cells. Endocrinology 1983;113:657-62.
13. Steiner AL, Parker CW, Kipnis DM. Radioimmunoassay for cyclic nucleotides. J Biol Chem 1972;247:1106-10.
14. Yagel S, Lala PK, Powell WA, Casper RF. Interleukin-1 stimulates human chorionic gonadotropin secretion by first trimester human trophoblast. J Clin Endocrinol Metab 1989;68:992-5.
15. Casey ML, Cox SM, Beutler B, Milewich L, MacDonald PC. Cachectin/tumor necrosis factor-α formation in human decidua. J Clin Invest 1989;83:430-436.
16. Jones SA, Challis JRG. Local stimulation of prostaglandin production by corticotropin-releasing hormone in human fetal membranes and placenta. Biochem Biophys Res Commun 1989;159:192-9.

Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep

Robert J. Morrow, MD, S. Lee Adamson, PhD, Shelley B. Bull, PhD, and J. W. Knox Ritchie, MD

Toronto, Ontario, Canada

The effect of hypoxic acidemia, hyperviscosity, and maternal hypertension on the umbilical arterial velocity waveform was studied in 23 chronically catheterized fetal sheep. Fetal hypoxic acidemia induced by lowering the maternal inspired oxygen concentration (n = 7) caused no change in the ratio of systolic/diastolic blood velocity even when fetal arteral pH was as low as 6.8. Fetal blood hyperviscosity (n = 7) induced by exchange transfusion with packed maternal blood cells increased placental vascular resistance by ≥50% but had no significant effect on the systolic/diastolic ratio. Similarly, maternal hypertension induced by intravenous infusion of angiotensin II to the ewe (n = 9) did not affect the systolic/diastolic ratio despite a 50% increase in maternal arterial blood pressure. We conclude that umbilical arterial velocity waveform abnormalities observed in growth-restricted human fetuses are probably not a direct result of fetal hypoxemia or hyperviscosity or maternal hypertension. (AM J OBSTET GYNECOL 1990;163:1313-20.)

Key words: Doppler ultrasonography, umbilical blood flow, intrauterine growth restriction, fetal assessment

Doppler ultrasonography is widely used in obstetrics to investigate blood velocity waveforms in the umbilical artery of human fetuses mainly because of the observation that the umbilical velocity waveform is often dramatically altered when fetal growth restriction is present.[1, 2] An abnormal velocity waveform is characterized by low, zero, or reversed blood velocity during diastole, relative to that during systole. Because abnormalities in the umbilical velocity waveform have been associated with poor neonatal outcome,[3, 4, 5] it has been suggested that the observation of an abnormal waveform should lead to a clinical intervention ranging from increased surveillance[6] to premature delivery of the fetus.[7] Despite these recommendations, it is not known how abnormal waveforms relate to the physiologic status of the fetus.

Pregnancies with fetal intrauterine growth restriction exhibit a higher incidence of hypoxia,[8, 9] hyperviscosity,[10] and pregnancy-induced hypertension,[11] and are more likely to have abnormal umbilical arterial velocity waveforms than are normally grown fetuses.[2] Increased placental vascular resistance has been shown to induce abnormal umbilical arterial velocity waveforms in fetal sheep[12, 13] and there is histologic evidence to suggest that placental resistance may be higher than normal in growth-restricted human fetuses.[14, 15] Thus elevated placental vascular resistance appears to play an important role in the induction of umbilical waveform abnormalities in growth-restricted fetuses. However, it is not known whether other factors could also cause umbilical arterial velocity waveform abnormalities. Because hypoxia, hyperviscosity, and maternal hypertension are more common in growth-restricted fetuses, we investigated whether these factors could play a role in the production of abnormal umbilical arterial waveforms.

From the Department of Obstetrics and Gynecology, University of Toronto, and Divisions of Perinatology and Clinical Epidemiology, Mount Sinai Hospital Research Institute.
This research was supported by grants from the Physician Services Incorporated and the Medical Research Council of Canada. R. J. M. is supported by the Hospital for Sick Children Foundation, and S. L. A. is a Scholar of the Medical Research Council of Canada.
Received for publication November 28, 1989; revised March 29, 1990; accepted June 1, 1990.
Reprint requests: Dr. R. J. Morrow, Department of Obstetrics and Gynecology, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada M5G 1X5.
6/1/22842

Material and methods

We developed and used a chronically catheterized fetal sheep preparation with Doppler crystals implanted on the umbilical artery for these studies. First we compared Doppler signals obtained with the implanted crystals with those obtained with a commonly used clincial Doppler machine to ensure that the signals obtained were relevant to the clinical situation. We then

performed experiments on three different groups of animals. In the first group, maternal hypoxia was used to induce fetal hypoxemia that was sufficient to cause progressive fetal acidosis. In the second group, fetuses were exchange transfused with packed maternal red blood cells until fetal blood viscosity was increased by ≥50%. In the third group, three dose levels of angiotensin II were infused to the ewe over 15 minutes to induce maternal hypertension.

General procedures. Anesthesia of pregnant ewes between 120 and 125 days' gestation was induced with thiopental sodium (1 gm administered intravenously) and was maintained with halothane and nitrous oxide in oxygen. The hind part of the fetus was withdrawn through a uterine incision and a midline incision was made in the fetal abdomen below the umbilicus to expose an umbilical artery. A flexible silicone cuff that contained a Doppler crystal mounted at approximately 45° to the direction of blood flow (20 MHz; Crystal Biotech, Halliston, Mass.) was sutured in place around one of the umbilical arteries. Vasospasm caused by manipulation of the artery was reduced by applying a muscle relaxant to the artery during surgery (papaverine hydrochloride, Frosst, Kirkland, Quebec, Canada). Two polyvinyl catheters were inserted into the femoral artery so that their tips lay in the aorta. They were used to sample blood for blood gas analysis, to withdraw samples containing radioactive microspheres, and to continuously monitor blood pressure. Another catheter was inserted into the inferior vena cava via the femoral vein and it was used to continuously monitor blood pressure and to infuse radioactive microspheres for flow measurements. Blood pressure levels were measured relative to amniotic pressure, which was monitored by means of a catheter sutured to the fetal abdomen.

Ewes were given an analgesic agent (Levo-Dromoran, levorphanol tartrate preparation, 2 mg subcutaneously) after surgery, and prophylactic antibiotics (Pen-di-Strep, Rogar, Canada) for 3 days postoperatively. Experiments were performed ≥5 days after surgery. Surgery and experiments were conducted in accordance with guidelines prescribed by the Canadian Council on Animal Care.

Vascular catheters were connected to pressure transducers (Model P23ID, Spectramed, Oxnard, Calif.) and the Doppler leads were connected to a Doppler flow meter (Model VF-1, Crystal Biotech). Blood pressure levels, heart rates, and umbilical arterial blood velocities were continuously monitored on a polygraph (Grass Instruments, Quincy, Mass.). Maternal and fetal arterial blood samples were obtained at intervals throughout the experiments and were measured immediately for blood gas and pH levels on a blood gas analyzer (Model 170, Corning Medical, Medfield, Mass.). Maternal and fetal bood gas values were temperature-corrected to 38° C and 39° C, respectively. Arterial oxygen content was measured with a co-oximeter (Model 2500, Corning Medical).

Placental blood flow was measured with the radioactive microsphere technique.[16] Immediately after starting collection of arterial reference samples with a syringe pump (2 ml · min^{-1} over 2.5 minutes), microspheres (15 μm, 3M, St. Paul, Minn.) labeled with scandium 46, cerium 141, chromium 51 or niobium 95 were introduced into the inferior vena cava over 20 seconds. Radioactivity in the placenta was counted (Model 1282 CompuGamma, LKB-Wallac, Turku, Finland) and flows were derived as previously described.[16] Placental vascular resistance was calculated by dividing the aorta to vena cava pressure gradient by the placental blood flow.

In all experiments a logarithmic transformation was performed on umbilical blood flow and placental vascular resistance to normalize their distributions. The analysis of the diastolic/systolic ratio, the inverse transformation of the systolic/diastolic ratio (S/D), gave identical conclusions so only the S/D ratio is presented. All data are presented as the mean ± SEM of the untransformed data.

Series I—validation of model (n = 6). After surgery, while ewes were still anesthetized, the S/D ratio was simultaneously measured from the umbilical artery with two instruments: our pulsed Doppler device that uses implanted crystals and a transcutaneous, continuous-wave Doppler instrument commonly used clinically (4 MHz; Multigon, Mount Vernon, N.Y.). The sample volume (1 mm^3) on the pulsed Doppler instrument was scanned across the vessel until the maximum velocity was obtained; thereafter the maximum velocity signal was monitored on an oscilloscope. The Doppler beam of the transcutaneous instrument was directed toward the umbilical arteries, which had been located before closure of the uterus, and the direction was then adjusted to obtain the characteristic umbilical waveform.[17] The Doppler signal from the transcutaneous device was continuously displayed on a video display screen. S/D ratios were measured from at least five cardiac cycles simultaneously frozen on the oscilloscope and the video display screen. Electronic calipers were used to measure the S/D ratio on the frozen video image obtained with transcutaneous Doppler ultrasonography. The S/D ratio from the implanted crystals was measured from a photograph of the oscilloscope screen.

Blood flow to the placenta was measured with radioactive microspheres (as described above) after at least 5 days' recovery from surgery. The flow distribution to each umbilical artery was measured to ensure that blood flow through this highly vasoactive vessel was not altered by the presence of the cuff. Four measurements

of blood flow were made in each of six fetuses. At postmortem examination, one umbilical artery was randomly selected and cannulated. Methylene blue dye was infused so that the cotyledons in the distribution of that artery were stained blue. Those in the distribution of the other artery remained unstained. The cotyledons that were only partly stained were excluded from this analysis (but were not excluded from the determination of total placental blood flow described above). Blood flow through each umbilical artery was calculated and expressed in milliliters per minute per 100 gm cotyledon wet weight.

Differences between the two Doppler methods and between the two umbilical artery blood flows were assessed with graphic techniques and estimates of the range of values in which 95% of differences in a single measurement are expected to lie.[18]

Series II—hypoxic acidemia (n = 7). An additional catheter (5 mm outer diameter) was inserted nonocclusively into the trachea of the ewe through a small incision below the fourth cricoid cartilage. Gas mixtures were passed through this catheter to manipulate maternal inspired oxygen concentration.[19]

During hypoxemia, the flow rate of nitrogen into the maternal tracheal catheter was adjusted to induce a 50% reduction in fetal arterial oxygen content. Carbon dioxide was added to the nitrogen infusion to maintain a constant arterial P_{CO_2}. During baseline measurements, before the induction of hypoxemia, air was infused at the same flow rate (about 16 L · min^{-1}) through the tracheal catheter. Fetal blood gas values and cardiovascular variables were measured three times at 5-minute intervals and blood flow was measured once during the baseline period. During hypoxemia, the gradual development of fetal acidemia was monitored by measurement of fetal blood gas and pH levels every 30 minutes. When the fetal pH reached 7.2, 7.0, and 6.8 the measurement protocol described during the baseline period was repeated. One fetus died between pH 7.2 and 7.0, two fetuses died between pH 7.0 and 6.8, and data are missing for one fetus at pH 7.0 because of technical reasons.

The data for each animal were summarized by averaging the three measurements made within each of four periods: baseline, pH ≤ 7.2, pH ≤ 7.0, and pH ≤ 6.8. Each of the averages at pH ≤ 7.2 and at pH ≤ 7.0 were compared with the baseline average with a paired Student t test. Statistical significance was then assessed at the 2.5% rather than the 5% level to control for multiple testing. Data obtained at pH ≤ 6.8 was not tested for significance because values reflected terminal changes and because the sample size was depleted (n = 2 or 4, depending on the variable).

Series III—fetal hyperviscosity (n = 7). Blood was collected from adult donor sheep into plastic blood bags (Fenwal, Division of Travenol Labs Inc., Deerfield, Ill.) containing citrate phosphate dextrose adenine solution and heparin. The bags were centrifuged and the plasma was removed.

Blood viscosity was measured at 39° C as a function of shear rate with a cone and plate viscometer (Wells-Brookfield, Brookfield Engineering Laboratories Inc., Stoughton, Mass.). Three baseline viscosity measurements were made on heparinized fetal blood drawn at 15-minute intervals. The packed red blood cells were then introduced into the inferior vena cava while blood was simultaneously withdrawn at the same rate from the fetal aorta (3 ml · min^{-1}; Harvard Apparatus, South Natick, Mass.). The transfusion was continued until the fetal blood viscosity was increased by ≥50%. After a 1-hour equilibration period, measurements were repeated at the elevated viscosity.

A paired Student t test was used to compare the average of the three baseline measurements with the average of the three measurements made at increased viscosity.

Series IV—maternal hypertension (n = 9). An additional vascular catheter was implanted in the maternal femoral artery so that blood pressure could be monitored.

Angiotensin II (Ciba Geigy, Toronto) in isotonic saline solution was infused for 15 minutes via the femoral vein of the ewe at doses of 5, 10, and 15 μg · min^{-1} at a flow rate of 15 ml · min^{-1}. These doses were chosen because a dose of 11.5 μg · min^{-1} had previously been shown to cause elevated maternal blood pressure and changes in the fetal circulation suggesting increased placental vascular resistance.[20] Isotonic saline solution infused at 15 ml · min^{-1} was used as a control. Each 15-minute infusion was followed by a 45-minute recovery period before the next dose was infused. The order of infusions was randomized.

Two measurements were made before each infusion (−10 and 0 minutes) and during each infusion (+10 and +15 minutes). The two values within each time period were similar so they were averaged to reduce variability in the data. The difference between measurements obtained before and during each infusion was used for subsequent statistical analysis. Repeated measures analysis of variance was used to determine the effect of different dosage levels of angiotensin II for animals with complete information for all infusions (n = 6), followed by paired Student t tests that incorporated all available information.

Results

Series I—validation of implanted crystals. The S/D ratios obtained from the umbilical artery with the internal and the external Doppler instruments were highly correlated (r = 0.98) and differences between

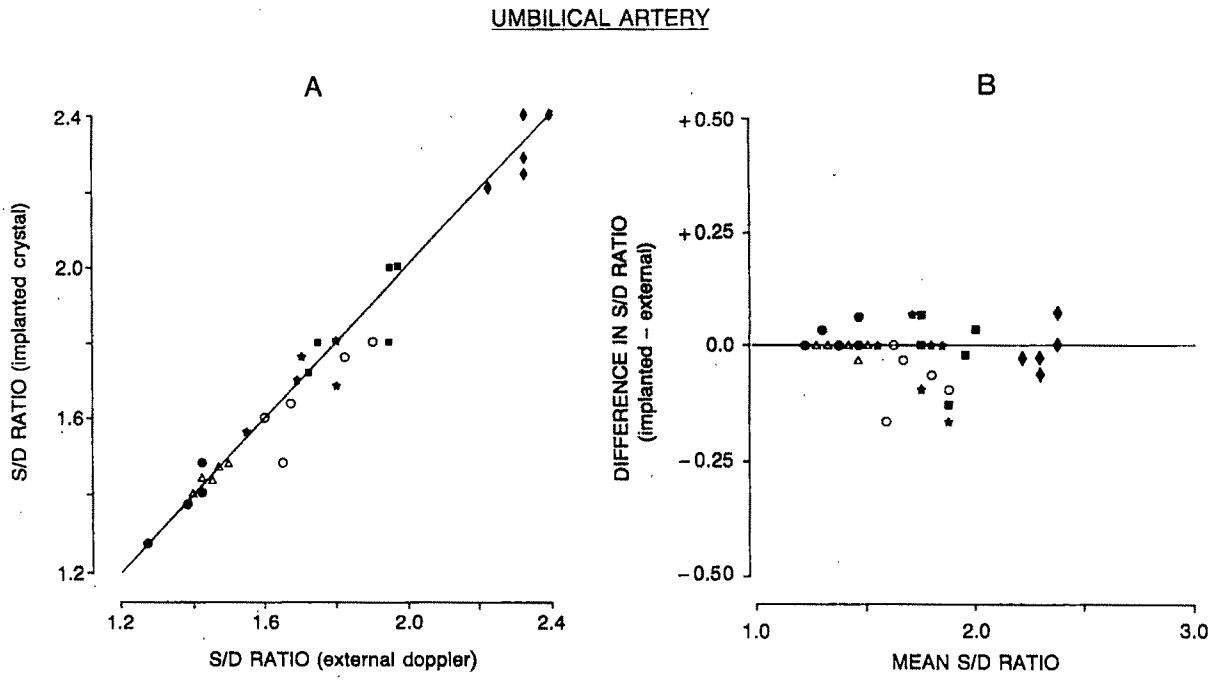


Fig. 1. A, Comparison of S/D ratio in umbilical artery with implanted Doppler crystal and clinical Doppler instrument (external). Each symbol represents individual animal. *Solid line* shows line of identity. **B,** Plot of differences between S/D ratio obtained with external and implanted Doppler techniques. That values lie close to zero throughout range of mean S/D ratio indicates there was no systematic difference between methods of measurement.

the two measurements were small (Fig. 1); 95% of the differences would be expected to be within ±0.16. The overall mean difference between the two signals was 0.02 and the size of the difference did not depend on the size of the ratio ($r = 0.11, p = 0.84$). Thus, the two methods of measurement were in close agreement.

The mean blood flow to the part of the placenta supplied by the artery monitored by the Doppler crystal was 145 ml · min^{-1} per 100 gm of placenta compared with 153 ml · min^{-1} per 100 gm of placenta in the other artery. The correlation coefficient between flows measured in each artery was 0.90 and, expressed as a percentage difference, the overall mean difference was 2.8% with 95% of individual differences expected to lie within ±20%. There was no significant difference between the weight of placenta supplied by each artery.

Series II—hypoxic acidemia. The mean fetal pH before induction of hypoxemia was 7.37 ± 0.01. The mean pH at each of the measurement points were 7.13 ± 0.02 (pH ≤ 7.2), 6.95 ± 0.02 (pH ≤ 7.0), and 6.75 ± 0.03 (pH ≤ 6.8). During hypoxemia, the oxygen content was reduced from 7.8 ± 0.3 ml · dl^{-1} to 2.8 ± 0.1 ml · dl^{-1} at the first measurement point (pH ≤ 7.2) and it remained at <50% of baseline for the duration of the hypoxic period (approximately 8 hours). The mean Pao_2 was 22 ± 1 mm Hg at baseline and it decreased to 11 ± 1 mm Hg at the first measurement point. The mean $Paco_2$ showed little change from the baseline value of 49 ± 2 mm Hg although it decreased slightly during hypoxemia ($Paco_2$ at the first measurement point was 44 ± 2 mm Hg).

The fetal arterial blood pressure, heart rate, and placental vascular resistance did not significantly alter with increasing acidosis (at pH ≤ 7.2 and pH ≤ 7.0). The systolic, diastolic, and mean umbilical velocities all showed small but significant decreases with progressive acidosis although the decrease in umbilical blood flow was not statistically significant (Table I). Nevertheless, the S/D ratio did not significantly change from the baseline value of 2.1 ± 0.1 with progressive acidosis down to pH ≤ 7.0 (Fig. 2). In all cases, however, we observed an increase in S/D ratio when hypotension and bradycardia developed in the fetus shortly before death. At pH ≤ 6.8, two of the four fetuses were hypotensive (arterial pressure levels were 28 mm Hg and 17 mm Hg) and bradycardic (heart rates were 108 and 80 min^{-1}) and both fetuses had zero diastolic velocities. The remaining two fetuses at pH ≤ 6.8 had arterial pressure levels of 44 and 27 mm Hg, heart rates within the normal range (153 and 172 min^{-1}), and S/D ratios of 3.7 and 3.8, respectively.

Series III—fetal hyperviscosity. Fetal hematocrit was 30% ± 3% before exchange transfusion with packed maternal blood cells. After transfusion the he-

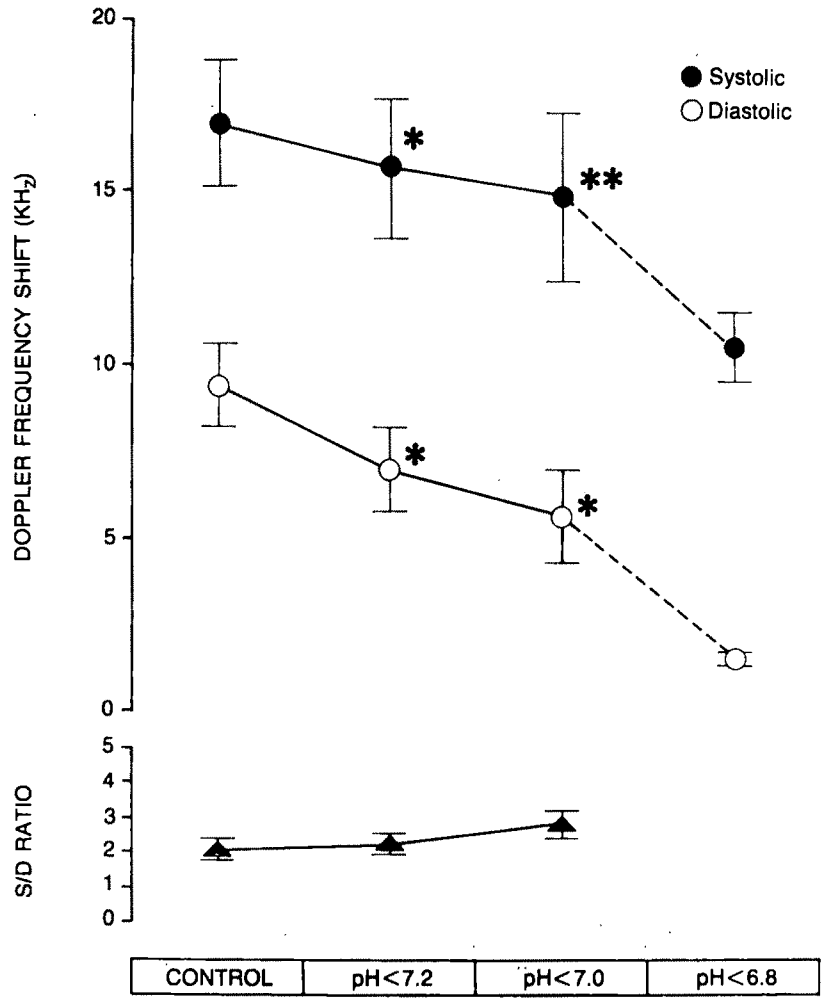


Fig. 2. Systolic and diastolic Doppler frequency shift (proportional to blood velocity) and S/D ratio are shown for both control and progressive acidemia. Only four of seven fetuses survived to pH ≤ 6.8 so data points are joined by *dashed lines* (these data were not subjected to statistical analysis). *$p \leq 0.025$ compared with control. **$p = 0.002$ compared with control.

matocrit was increased to 45% ± 2%. The mean blood viscosity at the three shear rates tested (90, 255, and 450 seconds^{-1}) was 2.3 ± 0.2 cP before and 3.8 ± 0.2 cP after transfusion. This increase caused a significant 50% increase in placental vascular resistance (Table II).

Hyperviscosity did not significantly affect the umbilical artery S/D ratio; however, the mean velocity and the diastolic velocity were both significantly reduced and the systolic velocity showed a decreasing trend ($p = 0.07$) (Table II). The mean fetal arterial pressure increased significantly but there was no significant change in fetal heart rate or umbilical blood flow. Fetal Pa_{O_2}, Pa_{CO_2}, oxygen content, and pH were not significantly altered from control values of 20 ± 1 mm Hg, 50 ± 2 mm Hg, 6.1 ± 0.4 ml · dl^{-1}, and 7.36 ± 0.01, respectively, by the exchange transfusion.

Series IV—maternal hypertension. Maternal mean arterial blood pressure was 91 ± 8 mm Hg during infusion of saline solution. During infusions of 10 and 15 μg · min^{-1} angiotensin II, maternal arterial blood pressure levels increased to 125 ± 5 and 136 ± 7 mm Hg, respectively. Mean maternal heart rate, P_{O_2}, oxygen content, P_{CO_2}, and pH were not significantly affected by angiotensin II infusions.

The umbilical S/D ratio during infusion of saline solution was 2.4 ± 0.2 and it did not significantly change during angiotensin II infusion at any dosage studied. There were no significant changes in mean fetal arterial blood pressure, heart rate, placental blood flow, placental vascular resistance, or blood gas and pH values during maternal infusions of angiotensin II.

Comment

Continuous monitoring of the umbilical arterial velocity waveform in the conscious animal was readily achieved with implanted Doppler crystals. We have

Table I. Fetal cardiovascular changes with increasing fetal acidemia (mean ± SEM)

		pH		
	Control	*<7.2*	*<7.0*	*<6.8*
Mean velocity (kHz)	12.4 ± 1.4	11.0 ± 1.6*	10.3 ± 1.9*	6.1 ± 1.7
Heart rate (min^{-1})	169 ± 5	173 ± 12	184 ± 9	128 ± 21
Mean arterial pressure (mm Hg)	40 ± 2	44 ± 3	37 ± 4	29 ± 6
PVR (mm Hg · ml^{-1} · min · kg^{-1})	0.23 ± 0.03	0.31 ± 0.08	0.21 ± 0.02	0.5 ± 0.2
UBF (ml · min^{-1} · kg^{-1})	174 ± 15	164 ± 30	150 ± 30	84 ± 33

PVR, Placental vascular resistance; *UBF*, umbilical blood flow. Diastolic velocity, systolic velocity, and S/D ratio are shown in Fig. 2.

*Statistically different from control, $p = 0.01$ for both comparisons.

Table II. The fetal cardiovascular parameters before and after exchange transfusion with packed maternal cells (mean ± SEM)

	Control	*Transfused*	*p Value*
PVR (mm Hg · ml^{-1} · min · kg^{-1})	0.24 ± 0.04	0.36 ± 0.06	0.03
Blood pressure (mm Hg)	41 ± 1	47 ± 2	0.005
Mean velocity (kHz)	14.5 ± 1.5	12.4 ± 1.5	0.04
Diastolic velocity (kHz)	10.4 ± 1.2	8.5 ± 1.5	0.05
Systolic velocity (kHz)	20.3 ± 1.3	18.1 ± 1.3	(0.07)
S/D ratio	2.1 ± 0.2	2.6 ± 0.5	(0.17)
Umbilical flow (ml · min^{-1} · kg^{-1})	181 ± 22	136 ± 20	(0.10)
Heart rate (min^{-1})	178 ± 8	170 ± 9	(0.96)

PVR, Placental vascular resistance.

demonstrated that waveforms recorded by this method are similar to those obtained with a transcutaneous, continuous-wave Doppler instrument. We have also shown that the presence of the cuff around the artery does not affect blood flow through this highly vasoactive vessel. Although the placental anatomy of the sheep differs from that of human beings we found that waveform shapes and S/D ratios in the sheep umbilical arteries were similar to those observed in the human fetus near term.[1, 17] Furthermore, the umbilical arterial S/D ratio in fetal sheep decreases with advancing gestational age[21] in a manner similar to that in human beings.[17] Thus the described fetal sheep preparation provides a suitable model for studying the cause of abnormal umbilical arterial waveforms.

We have shown that hypoxic acidosis down to a pH of 7.0 does not cause a significant change in the umbilical arterial velocity waveform. This suggests that redistribution of blood flow that occurs in response to hypoxic acidosis[22] does not affect the umbilical arterial waveform. However, the S/D ratio ultimately increased in all fetuses when the pH ≤ 6.8. This may have been mediated by a decrease in myocardial contractility caused by acidemia.[23] Although the mechanism is not known, diastolic velocity decreased to zero in all fetuses when hypotension and bradycardia occurred before fetal death. Because changes in the S/D ratio were only marked when the fetus was near death, the umbilical artery velocity waveform does not appear to provide an early warning of developing fetal hypoxic acidosis. This finding is in agreement with Weiner[24] who studied the relationship between umbilical arterial S/D ratio and the umbilical venous acid-base status in human fetuses and concluded that an abnormal umbilical arterial S/D ratio was not a sufficiently accurate indicator of fetal acid-base status to form the basis for clincial management.

We have increased viscosity by 60% and have observed no change in the umbilical waveform shape as measured by the S/D ratio. This result indicates that it is highly unlikely that the association between the much smaller elevation in blood viscosity and abnormal umbilical arterial velocity waveforms observed by Giles and Trudinger[25] in human subjects was a direct cause-effect relationship.

Umbilical arterial waveforms and fetal placental resistance did not change even during large increases in maternal arterial blood pressure induced by maternal angiotensin II infusion. Pressure in the maternal blood vessels of the sheep placenta influence fetal placental vascular resistance presumably by causing partial collapse of the fetal placental vessels.[26] Thus we hypothesized that an increase in maternal arterial blood pressure could increase fetal placental vascular resistance and that this effect could play a role in producing the umbilical arterial waveform abnormalities observed in

patients with pregnancy-induced hypertension. Because no significant change in umbilical vascular resistance was observed, it is possible that the increase in central arterial pressure was not transmitted to the small exchange vessels of the maternal placenta. Whether high central pressures are transmitted to the placental microcirculation in hypertensive pregnant women is not known. The absence of a direct effect of maternal blood pressure on umbilical waveforms may explain why abnormal waveforms are often not observed in women with chronic hypertension or mild pregnancy-induced hypertension.[27]

The absence of significant fetal cardiovascular effects during maternal angiotensin II infusion is consistent with studies that concluded that angiotensin II does not cross the placenta in an active form[28, 29] but contradict those of Perales et al.[20] Although we used a similar dose of angiotensin II and induced a similar increase in maternal arterial blood pressure, we were unable to reproduce the significant increase in fetal blood pressure and decrease in heart rate described by Perales et al.[20] The reason for the discrepancy in results is not known.

There is growing evidence that the umbilical S/D ratio reflects placental vascular resistance.[12, 13, 30] However, we caused a substantial increase in the placental vascular resistance by increasing blood viscosity but the S/D ratio was not significantly altered. The 50% increase in resistance should have been sufficient to produce an effect if the waveform were sensitive to viscosity inasmuch as a 40% increase in placental vascular resistance caused by placental embolization had a significant effect.[30] It is noteworthy that, like viscosity, an increase in placental vascular resistance caused by umbilical arterial vasoconstriction also has no effect on the umbilical arterial velocity waveform.[13] Thus, the mechanism by which placental vascular resistance is increased is important in determining whether waveform shape is affected.

In conclusion, we have demonstrated that hypoxic acidemia in the sheep fetus does not significantly affect the umbilical arterial velocity waveform until fetal death is imminent. Thus a fetus may be hypoxic and acidotic, but still have a normal waveform if the placental ultrastructure is normal. Normal waveforms are also observed when resistance to fetal placental blood flow is increased either by hyperviscosity or by vasoconstriction of the umbilical arteries.[13] On the other hand, when peripheral placental resistance is elevated by embolization with microspheres, the waveform abnormalities observed in growth-restricted human fetuses are reproduced.[12, 18] Thus apart from cardiovascular collapse before death, it appears that an abnormal umbilical arterial velocity waveform is a very specific indicator of elevated resistance within the small vessels of the fetal placenta and should be used with caution as a more general indicator of fetal condition.

We thank Dr. C. E. Musclow for her assistance in preparing packed blood cells and for measuring fetal blood viscosity.

REFERENCES

1. Erskine RLA, Ritchie JWK. Umbilical artery blood flow characteristics in normal and growth retarded fetuses. Br J Obstet Gynaecol 1985;92:605-10.
2. Fleischer A, Schulman H, Farmakides G, et al. Umbilical velocity waveforms in intrauterine growth retardation. Am J Obstet Gynecol 1985;151:502-5.
3. McCowan LM, Erskine LA, Ritchie JWK. Umbilical artery Doppler blood flow studies in the preterm small-for-gestational-age fetus. Am J Obstet Gynecol 1987;156: 655-9.
4. Laurin J, Lingman G, Marsal K, Persson P-H. Fetal blood flow in pregnancies complicated by intrauterine growth retardation. Obstet Gynecol 1987;69:895-902.
5. Al-Ghazali W, Chapman MG, Allan LD. Doppler assessment of the cardiac and uteroplacental circulations in normal and complicated pregnancies. Br J Obstet Gynaecol 1988;95:575-80.
6. Ombelet W, Nuradi S, Vandenberghe K, Spitz B, Van Assche A. Absent or reversed end-diastolic flow in the umbilical arteries: a warning sign of serious fetal compromise. Clin Exp Hypertens Preg 1988;B7:303-16.
7. Woo JSK, Liang ST, Lo RLS. Significance of an absent or reversed end-diastolic flow in Doppler umbilical artery waveforms. J Ultrasound Med 1987;6:291-7.
8. Soothill PW, Bilardo CM, Nicolaides KH, Campbell S. Relation of fetal hypoxia in growth retardation to mean blood velocity in the fetal aorta. Lancet 1986;2:1118-9.
9. Nicolaides KH, Bradley RJ, Soothill PW, Campbell S, Bilardo CM, Gibb D. Maternal oxygen therapy for intrauterine growth retardation. Lancet 1987;1:942-5.
10. Hakanson DO, Oh W. Hyperviscosity in the small-for-gestational age infant. Biol Neonate 1980;37:109-12.
11. Rochelson BL, Schulman H, Fleischer A, et al. The clinical significance of Doppler umbilical artery velocimetry in the small-for-gestational-age fetus. Am J Obstet Gynecol 1987;156:1223-6.
12. Morrow RJ, Adamson SL, Bull SB, Ritchie JWK. Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep. Am J Obstet Gynecol 1989; 161:1055-60.
13. Adamson SL, Morrow RJ, Langille BL, Bull SB, Ritchie JWK. Site-dependent effects of increases in placental vascular resistance on the umbilical arterial velocity waveform in fetal sheep. Ultrasound Med Biol 1990;16:19-27.
14. Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. Br J Obstet Gynaecol 1985;92:31-8.
15. McCowan LM, Mullen BM, Ritchie JWK. Umbilical artery flow velocity waveforms and the placental vascular bed. Am J Obstet Gynecol 1987;157:900-2.
16. Heymann MA, Payne BD, Hoffman JIE, Rudolph AM. Blood flow measurements with radionuclide-labeled particles. Prog Cardiovasc Dis 1977;20:55-79.
17. Schulman H, Fleischer A, Stern W, et al. Umbilical velocity wave ratios in human pregnancy. Am J Obstet Gynecol 1984;148:985-90.
18. Bland MJ, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-10.
19. Gleed RD, Poore ER, Figueroa JP, Nathanielz PW. Modification of maternal and fetal oxygenation with the use of tracheal gas infusion. Am J Obstet Gynecol 1986; 155:429-38.

20. Perales AJ, Naden RP, Laptook A, Rosenfeld CR. Fetal response to maternal infusions of angiotensin II. Am J Obstet Gynecol 1986;154:195-203.
21. Newnham JP, Kelly RW, Roberts RV, et al. Fetal and maternal Doppler flow velocity waveforms in normal sheep pregnancy. Placenta 1987;8:467-76.
22. Cohn HE, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. Am J Obstet Gynecol 1974;120:817-24.
23. Fisher DJ. Acidaemia reduces cardiac output and left ventricular contractility in conscious lambs. J Dev Physiol 1986;8:23-31.
24. Weiner CP. Pathogenesis, evaluation, and potential treatments for severe, early onset growth retardation. Semin Perinatol 1989;13:320-7.
25. Giles WB, Trudinger BJ. Umbilical cord whole blood viscosity and the umbilical artery flow velocity waveform: a correlation. Br J Obstet Gynaecol 1986;93:466-70.
26. Power GG, Longo LD. Sluice flow in placenta: maternal vascular pressure effects on fetal circulation. Am J Physiol 1973;255:1490-6.
27. Cameron AD, Nicholson SF, Nimrod CA, Harder JR, Davies DM. Doppler waveforms in the fetal aorta and umbilical artery in patients with hypertension in pregnancy. Am J Obstet Gynecol 1988;158:339-45.
28. Bruce SL, Morishima HO, Petrie RH, et al. Response of ovine uterine blood flow to angiotensin II: effect on the fetus. Am J Obstet Gynecol 1981;141:495-8.
29. Pipkin FB, Benjamin N, MacAllan C. Placental transfer of a large angiotensin fragment in the guinea pig. Am J Obstet Gynecol 1977;128:904-6.
30. Trudinger BJ, Stevens D, Connelly A, et al. Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation. Am J Obstet Gynecol 1987;157:1443-8.

A progesterone-induced blocking factor corrects high resorption rates in mice treated with antiprogesterone

Julia Szekeres-Bartho, MD, PhD,[a] G. Chaouat, MD, PhD,[b] and R. Kinsky, DV, PhD[b]

Pecs, Hungary, and Paris, France

Earlier we showed that because of the presence of functional progesterone receptors, lymphocytes of healthy pregnant women produced an immunomodulatory protein in the presence of progesterone, whereas those of nonpregnant persons did not. Progesterone-treated murine pregnancy lymphocytes release a similar factor. The present study reveals the biologic significance of this finding. Treatment of BALB/c mice that were 8 days pregnant with a progesterone receptor blocker (RU 486) resulted in 100% resorption of the fetuses. Simultaneous administration of the supernatant from progesterone-treated murine pregnancy spleen cells restored the resorption rate to the original 6% observed in untreated control animals. These data suggest that functional lymphocytic progesterone binding sites are needed for the maintenance of normal pregnancy. Because of the blockage of progesterone receptors and the consequent inability of the lymphocytes to produce the progesterone-induced blocking factor, abortion is initiated by immune factors. The fact that administration of the preformed blocking factor counteracted the effect of antiprogesterone treatment suggests that progesterone-mediated immunosuppression is needed for the maintenance of normal gestation. (Am J Obstet Gynecol 1990;163:1320-2.)

Key words: Progesterone receptor block, abortion, progesterone-dependent immunomodulation

There is now ample evidence that the maternal immune system recognizes a semiallogeneic conceptus.[1, 2] Local mechanisms can in turn prevent potentially harmful antifetal immune reactions, thus assuring a normal outcome of pregnancy.[3] Progesterone is essential for the maintenance of mammalian gestation. In general, estrogens promote and progesterone inhibits uterine contractility, interfering in this way with the initiation of labor. Progesterone further affects in vitro lymphocyte reactivity.[4] We have shown that human pregnancy lymphocytes (unlike nonpregnancy ones) possess specific progesterone binding sites.[5, 6] When exposed to progesterone, these lymphocytes release a protein[7] endowed with diverse immunomodulatory properties.[8]

This protein appears in the serum of pregnant women and its concentration shows a correlation with

From the Institute of Microbiology, University Medical School,[a] and Institut National de la Santé et de la Recherche Medicale.[b]
Received for publication November 13, 1989; revised May 7, 1990; accepted June 2, 1990.
Reprint requests: J. Szekeres-Bartho, MD, PhD, Institute of Microbiology, University Medical School, Pecs, Hungary H-7643.
6/1/22866

Table I. The progesterone-induced blocking factor corrects the high resorption rate induced by antiprogesterone treatment in BALB/c mice that were 8 days pregnant

Group	Treatment	No. pregnant females	No. embryos			Mean litter size
			Living	Resorbed		
				n	%	
1	Injected with 2 mg/kg RU 486	4	0	12	100	3
2	Injected with 2 mg/kg RU 486 and 0.5 ml supernatant of progesterone-treated pregnancy spleen cells	4	15	1	6.2*	4
3	Injected with 2 mg/kg RU 486 and 0.5 ml supernatant of untreated pregnancy spleen cells	4	0	11	100	2.75
4	Injected with 2 mg/kg RU 486 and 0.5 ml supernatant of progesterone-treated spleen cells from virgin mice	4	0	13	100	3.25
5	—	11	62	8	11	6.36

*Significantly different from RU 486-treated mice at $p < 0.001$.

the success of pregnancy.[9] RU 486 mifepristone is a competitive antagonist of progesterone that blocks progesterone and to a certain extent glucocorticoid binding sites, thus leading to abortion in the early phase of pregnancy.[10] Data obtained by testing lymphocyte function of women treated with the progesterone receptor blocker ZK98734 suggests that the block of lymphocyte progesterone receptors correlates better with the abortive effect than the block of trophoblastic receptors.[11]

On the basis of these data we assume that progesterone-mediated immunosuppression is crucial for the maintenance of pregnancy. We present data that show a possibility to overcome receptor blocking by supplying in vivo the progesterone-induced immunomodulatory substance lacking in RU 486—treated pregnant females.

Material and methods

Production of the progesterone-induced blocking factor. C3H mice that were 12 days pregnant (Institute de la Recherche Scientifique sur le Cancer [IRSC], Villejuif, France) were killed and the spleens were harvested. Lymphocytes were isolated in Ficoll-Paque gradient, washed three times, and treated at a cell density of 5×10^6 viable cells per milliliter with 10 μg/ml of progesterone for 3 hours. At the end of the incubation period, supernatants were collected and dialyzed overnight at 4° C against 500× volume medium to eliminate progesterone. Radioimmunologic determination of progesterone revealed that almost 100% of the progesterone was depleted from the supernatants by dialysis.

Supernatants of progesterone-treated lymphocytes from virgin C3H mice and those of untreated pregnancy spleen cells were used as controls.

Treatment of pregnant mice with RU 486 and the progesterone-induced blocking factor. BALB/c mice were obtained from the IRSC breeding center (Centre National de la Recherche Scientifique, Villejuif, France). Successful matings were checked daily by vaginal plug examinations, and mated females were randomly separated into experimental and control groups.

At 8 days' gestation 2 mg/kg RU 486 was administered intraperitoneally alone or with 0.5 ml of supernatant of progesterone-treated pregnancy spleen cells or control supernatants. Experimental and control female mice were killed on day 10 of pregnancy and living and resorbing embryos were counted.

Statistical analysis. The data were analyzed by the χ^2 test.

Results

Treatment of BALB/c mice that were 8 days pregnant with a single 2 mg/kg dose of RU 486 resulted in 100% resorption of the fetuses. Simultaneous intraperitoneal administration of 0.5 ml of the supernatant of progesterone-treated pregnancy spleen cells prevented the abortive effect of the antiprogesterone (i.e., it restored the resorption rate to the original 6% observed in untreated control animals). The supernatants of untreated pregnancy spleen cells and those of progesterone-treated spleen cells from virgin mice were without effect (Table I).

Comment

Progesterone has been shown to exert immunologic effects in vitro.[4] This hormone is produced in large quantities during pregnancy and reaches high concentrations locally at the fetomaternal interface, thus it might be involved in local immunosuppression. Earlier we showed that progesterone-treated lymphocytes of healthy pregnant women produce a 34 kd immuno-

modulatory protein.[7] The in vitro effects of this substance include suppressor cell generation and inhibition of natural killer and natural cytotoxic activity.[8] The concentration of this factor was significantly higher in the sera of healthy pregnant women than that in the sera of nonpregnant persons or pregnant women with clinical symptoms of threatened abortion or threatened preterm delivery.[9]

The termination of pregnancy was found to be associated with increased natural cytotoxic activity of the lymphocytes.[12] The serum concentration of the blocking factor correlated well with the outcome of pregnancy, as well as with the natural killer blocking potential of the sera.[9] Therefore it seemed likely that the in vivo action of the protein involved blockage of natural killer activity and that the blockade of natural killer activity was needed for the maintenance of normal pregnancy. Natural killer activity might be involved in fetal tissue destruction as well as the blockage of blood supply and the induction of smooth muscle contraction by some of their secreted products (e.g., tumor necrosis factor).[13] This hypothesis was further supported by the fact that administration of the blocking factor significantly reduced the high resorption rate induced by the transfer of natural killer cells to pregnant BALB/c mice (unpublished observation).

Recently we demonstrated progesterone receptors in lymphocytes of healthy pregnant women but not in those of healthy nonpregnant persons.[5] Blockage of progesterone receptors both in human beings and animal models results in the cessation of pregnancy,[10] and the production of the blocking factor by human lymphocytes is progesterone receptor dependent.[5, 8]

Recently we showed in a study with human beings that at a certain dose progesterone receptor blockers affected trophoblastic and lymphocytic binding sites to a different extent, and that the involvement of lymphocytic progesterone receptors showed a higher degree of correlation with the success or failure of the treatment than that of the trophoblastic receptors.[11]

This study shows that administration of the preformed progesterone-induced blocking factor counteracts the abortive effect of progesterone receptor block. These data support the following hypothesis: normal pregnancy lymphocytes express specific binding sites for progesterone. This allows the production of the blocking factor when lymphocytes are exposed to the hormone. In the murine model the abortive effect of progesterone receptor block was overcome by the suppressive substance. The present study shows that the progesterone-induced immunologic blocking factor in addition to its in vitro immunomodulatory effects is able to substitute the immunologic effects of progesterone, and that this alone could alter the resorption rate in progesterone-deprived animals. Therefore it seems likely that progesterone-mediated immunosuppression has a role in the maintenance of gestation.

REFERENCES

1. Smith G. In vitro susceptibility of mouse placental trophoblast to cytotoxic effector cells. J Reprod Immunol 1983;5:39-44.
2. Chaouat G, Kolb J-P, Wegmann TG. The murine placenta as an immunologic barrier between the mother and the fetus. Immunol Rev 1983;75:31-60.
3. Stites DP, Pavia CS, Clemens LE, Kuhn RW, Siiteri PK. Immunologic regulation in pregnancy. Arthritis Rheum 1979;22:1300-4.
4. Stites DP, Siiteri PK. Steroids as immunosuppressants in pregnancy. Immunol Rev 1983;75:117-38.
5. Szekeres-Bartho J, Szekeres GY, Debre P, Autran B, Chaouat G. Reactivity of lymphocytes to a progesterone receptor-specific monoclonal antibody. Cell Immunol 1990;125:273-83.
6. Szekeres-Bartho J, Reznikoff-Etievant MF, Varga P, Varga Z, Chaouat G. Lymphocytic progesterone receptors in human pregnancy. J Reprod Immunol 1989;16:239-47.
7. Szekeres-Bartho J, Kilar F, Falkay G, Csernus V, Torok A, Pacsa AS. Progesterone-treated lymphocytes of healthy pregnant women release a factor inhibiting cytotoxicity and prostaglandin synthesis. Am J Reprod Immunol Microbiol 1985;9:15-8.
8. Szekeres-Bartho J, Autran B, Debre P, Andreu G, Denver L, Chaouat G. Immunoregulatory effects of suppressor factor from healthy pregnant women's lymphocytes after progesterone induction. Cell Immunol 1989;122:281-94.
9. Szekeres-Bartho J, Varga P, Pejtsik B. ELISA test for detecting an immunological blocking factor in human pregnancy serum. J Reprod Immunol 1989;16:19-23.
10. Philibert D, Moguilewsky M, Mary I, et al. Pharmacological profile of RU 486 in animals. In: Baulieu E-E, Segal SJ, eds. The antiprogestin steroid RU 486 and human fertility control. New York: Plenum Press, 1985:49-68.
11. Szekeres-Bartho J, Szabo J, Kovacs L. Alteration of lymphocyte reactivity in pregnant women treated with the progesterone receptor inhibitor ZK98734. Am J Reprod Immunol 1989;21:46-9.
12. Szekeres-Bartho J, Varga P, Pacsa AS. Immunological factors contributing to the initiation of labor—lymphocyte reactivity in term labor and threatened preterm delivery. AM J OBSTET GYNECOL 1986;155:108-12.
13. Warner JSC, Libby P. Human vascular smooth muscle. J Immunol 1989;142:100-3.

Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy

Kristjar Skajaa, MD,[a]* Danny Svane, MD,[a] Karl-Erik Andersson, MD, PhD,[b] and Axel Forman, MD, PhD[a]

Århus, Denmark, and Lund, Sweden

The effects of Mg^{++} and the dihydropyridine calcium channel blocker isradipine on contractions induced by the thromboxane A_2 mimetic U46619 were studied in isolated human uteroplacental arteries. In all preparations investigated U46619 10^{-10} to 10^{-6} mol/L induced concentration-related contractile responses. In maternal vessels U46619 produced a biphasic concentration-response curve, whereas the compound in fetal vessels produced a sigmoid concentration-response curve parallel to that of prostaglandin $F_{2\alpha}$. Mg^{++} (1.0 to 6.0 mmol/L) and isradipine (10^{-10} to 10^{-5} mol/L) both relaxed U46619-induced contractions by up to about 40%. In preparations precontracted by U46619 and exposed to isradipine 10^{-6} mol/L, addition of Mg^{++} 6.0 mmol/L consistently produced a further small relaxation. Removal of the endothelium or pretreatment with indomethacin 10^{-6} mol/L or digoxin 10^{-6} mol/L did not influence the inhibition produced by Mg^{++} or isradipine. In vessels pretreated in Ca^{++}-free medium Mg^{++} 6.0 mmol/L depressed and isradipine abolished responses to Ca^{++} (0.01 to 4.0 mmol/L) after depolarization with K^+ 124 mmol/L. In maternal and fetal arteries stimulated with U46619, however, Mg^{++} 6.0 mmol/L and isradipine 10^{-6} mol/L produced a similar, partial inhibition of responses to Ca^{++} (0.01 to 4.0 mmol/L). Mg^{++} seems to inhibit transmembrane Ca^{++} influx, although less efficiently than the dihydropyridine calcium antagonist isradipine. In addition, Mg^{++} may interfere with Ca^{++} at intracellular binding sites. Provided that preeclampsia comprises enhanced vascular actions of thromboxane A_2, the present results support the established use of Mg^{++} in the treatment of this condition and suggest that calcium antagonists are of potential benefit. (AM J OBSTET GYNECOL 1990;163:1323-33.)

Key words: Mg^{++}, calcium channel blocker, uteroplacental vessels, preeclampsia, thromboxane A_2

Increased peripheral vascular resistance and blood pressure with impaired uteroplacental blood flow are main characteristics of preeclampsia.[1, 2] These changes have been explained by imbalance in the synthesis pattern of prostacyclin and thromboxane A_2 (TxA_2). Although the role of prostacyclin has been questioned by results from infusion studies in vivo,[3] placental tissues show increased capacity to produce TxA_2,[4] which could account for the increased vascular resistance. Analogues of TxA_2 produce vasoconstriction in fetal placental vessels,[5] but the effects on the maternal uteroplacental vessels and the possibilities of interference with such responses are not known.

Magnesium is currently used in the treatment of preeclampsia, and the ion produces relaxation of various peripheral vessels,[6] including the fetal umbilical and stem villous arteries.[7, 8] The mechanisms involved in these responses seem to include interference with transmembrane Ca^{++} influx[6, 8] but may include enhanced endothelial production of prostacyclin as well.[9] Calcium channel blockers of the dihydropyridine group show antihypertensive effects in patients wtih preeclampsia,[10, 11] and these drugs produce relaxation of isolated uteroplacental arteries.[12] The effects of Mg^{++} and dihydropyridine calcium channel blockers on contractile activation induced by TxA_2 do not seem to have been studied in maternal and fetal uteroplacental arteries.

In this study we have tested the effects of the stable TxA_2 analog U46619 on isolated, small human uteroplacental arteries from term pregnant patients. The responses have been compared with those produced by norepinephrine and vasopressin in maternal vessels and those produced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in

From the Department of Obstetrics and Gynecology, University of Aarhus,[a] and the Department of Clinical Pharmacology, University of Lund.[b]
This investigation was supported by a grant from The Danish Medical Research Council.
Received for publication December 11, 1989; revised April 23, 1990; accepted June 2, 1990.
Reprint requests: Kristjar Skajaa, MD, Department of Obstetrics and Gynecology, University of Aarhus, 8000 Århus C, Denmark.
**Recipient of an award from Novo Industri Foundation, Copenhagen, Denmark.*
6/1/22869

fetal vessels. Moreover, we have studied the effects of Mg^{++} and isradipine on U46619-induced contractile activation in both types of vessel.

Material and methods

After informed, written consent was obtained, myometrial biopsy specimens were taken from the upper edge of the isthmic incision at term cesarean section because of genital herpes ($n = 2$), previous cesarean section ($n = 2$), or breech presentation ($n = 4$). In addition, macroscopically normal cotyledons were excised together with adjacent chorionic plate. All specimens were immediately placed in ice-cold Krebs solution and transported to the laboratory. By careful dissection under a stereomicroscope, stem villous arteries (inner diameter 300 to 400 μm) were identified in the cotyledons, and intramyometrial arteries (inner diameter 300 to 500 μm) were exposed in the myometrial biopsy specimens. The vessels were prepared as vascular rings (length 1.0 to 1.2 mm), and the preparations were then mounted in 5 ml organ baths containing Krebs solution (37.0° ± 0.5° C) bubbled with carbogen (95% oxygen and 5% carbon dioxide). The final pH was 7.40 ± 0.05. The short parallel legs of two L-shaped hooks were carefully inserted through the lumina of the vessels.[13] One of these hooks was attached to a displacement device that allowed fine adjustments of tension, and the other was connected to a Grass Ft 03 transducer. Isometric tension was recorded and displayed on a Beckmann R 611 polygraph.

The preparations were allowed to equilibrate for 1 hour. During this period passive tension was adjusted to 4 mN in the intramyometrial vessels and to 3 mN in the stem villous arteries. These resting tension levels were shown to be near optimum for contractile responses to depolarization with K^+ (124 mmol/L). After the equilibration period, contractions were repeatedly induced by K^+ (124 mmol/L) until reproducible (<10% variation between two successive contractions). Subsequent contractile responses were expressed as a percent of the mean of the last two of the initial K^+-induced contractions. The integrity of the endothelium was regularly controlled by testing the relaxant effects of substance P 10^{-7} mol/L in vessels precontracted by norepinephrine 10^{-5} mol/L (maternal vessels) or $PGF_{2\alpha}$ 10^{-5} mol/L. Only preparations showing more than 80% relaxation were used for further experiments. By contrast, in fetal and maternal vessels that were denuded of endothelial lining by gently moving a hair through the lumen, substance P induced <15% relaxation.

The relaxant effects of Mg^{++} and isradipine on U46619-induced tension were calculated by computerized planimetry during the 10-minute period subsequent to addition of the substance and expressed as a percentage of the area under the U46619-induced contraction 10 minutes before addition of the relaxing substances. Solvent-treated preparations contracted by U46619 served as controls.

Concentration-response relations to U46619, norepinephrine, vasopressin, and Ca^{++} were characterized by the maximum amplitude achieved (E_{max} in percent of initial K^+ response) and the concentration producing half-maximum response (EC_{50}). EC_{50} values were expressed as $pD_2 = -\log(EC_{50})$. Mean and SE for E_{max} and pD_2 were determined. After homogeneity of variance was ascertained, significance of difference was assessed by one-way analysis of variance or, if appropriate, by the two-tailed t test for groups of nonpaired observations with a level of significance of 0.05.

Solutions. Normal Krebs solution: sodium chloride, 119 mmol/L; potassium chloride, 4.6 mmol/L; sodium bicarbonate, 15 mmol/L; calcium chloride, 1.5 mmol/L; magnesium chloride, 1.2 mmol/L; sodium phosphate, 1.2 mmol/L; glucose, 11 mmol/L. High-K^+ Krebs solution: potassium chloride, 124 mmol/L; sodium bicarbonate, 15 mmol/L; calcium chloride, 1.5 mmol/L; magnesium chloride, 1.5 mmol/L; sodium phosphate, 1.2 mmol/L; glucose, 11 mmol/L. In Ca^{++}-free solutions calcium chloride was omitted and ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA) 0.01 mmol/L was added.

Drugs. Drugs used were as follows: U46619 [(15S)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid (Upjohn), isradipine (Sandoz), NA [(−)-norepinephrine, Sigma Chemical Co.], vasopressin (arginine vasopressin, Sigma), and $PGF_{2\alpha}$ (Dinoprost, Upjohn).

Results

Neither the maternal intramyometrial preparation nor the fetal stem villous arterial preparation exhibited spontaneous contractile activity. In maternal arteries K^+ (124 mmol/L) depolarization induced an initial fast rise in tension that developed into a tonic contraction with higher amplitude. The maximum tension reached was 10 ± 1 mN/mm ($n = 8$).

In the fetal vessels the initial rapid phase continued into a maintained contraction with lower amplitude within 1 to 2 minutes. The maximum amplitude was 10 ± 2 mN/mm ($n = 8$). The corresponding tension level reached during the second phase was 90% of the initial maximum amplitude.

Effects of U46619, norepinephrine, and vasopressin. U46619 10^{-10} to 10^{-6} mol/L, norepinephrine 10^{-8} to 10^{-4} mol/L, and vasopressin 10^{-11} to 10^{-8} mol/L all induced concentration-related contractile responses in the maternal intramyometrial vessels (Fig. 1). However, the shape of the concentration-response curves for

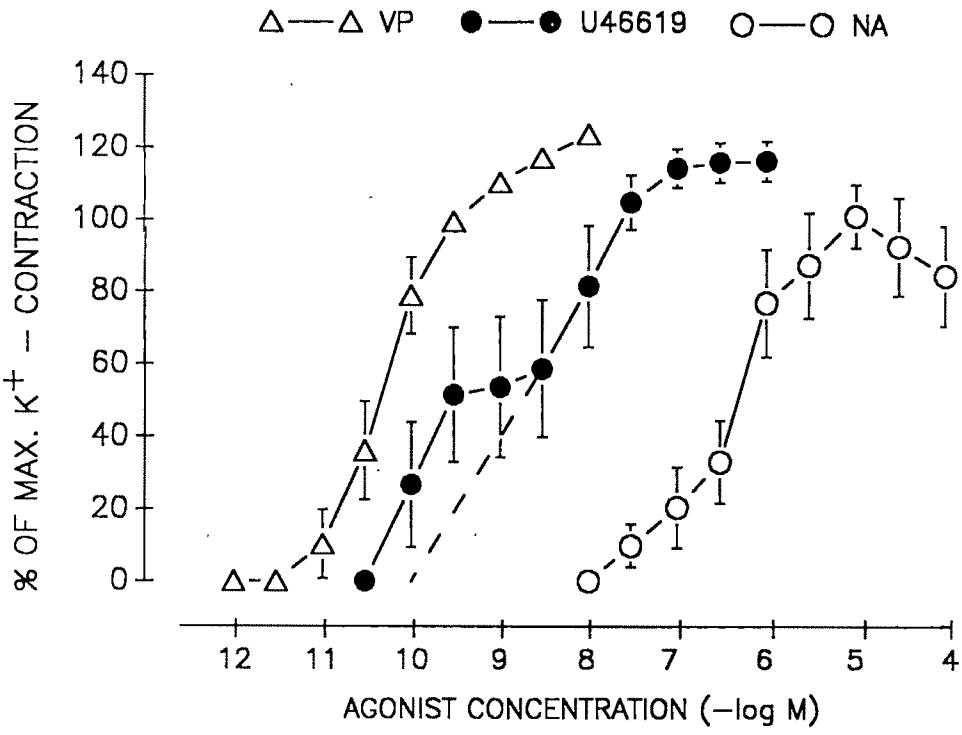


Fig. 1. Maternal intramyometrial arteries. Concentration-response relations for vasopressin *(VP)* 10^{-11} to 10^{-8} mol/L, U46619 10^{-10} to 10^{-6} mol/L, and NA *(NA)* 10^{-8} to 10^{-4} mol/L. Each point represents the mean of eight experiments. *Vertical bars* denote SE, when this exceeded the size of the symbols. The *dotted line* represents the linear extension of the latter part of the U46619 concentration-response curve, which was sued for calculation of the pD_2 value for that part of the U46619 response. See text for further details.

these agonists differed. Thus cumulative addition of U46619 produced a biphasic concentration-response curve. A plateau at about 50% of the K^+ response was achieved in the U46619 concentration range of 3×10^{-10} mol/L. The first part of the concentration-response curve had an apparent E_{max} of about 54% of the K^+ response with a corresponding pD_2 value of 9.6 ± 0.2. At further addition of U46619 a final E_{max} of 116% ± 6% of the K^+ response was reached. A pD_2 value of 8.5 ± 0.6 for this part of the curve was assessed by extrapolation of the linear part of the concentration-response curve (corresponding to U46619 3×10^{-9} to 3×10^{-8} mol/L) (Fig. 1). By contrast norepinephrine and vasopressin produced sigmoid concentration-response curves with a linear course corresponding to the EC_{50} value. E_{max} values of vasopressin- and norepinephrine-induced contractions were 124% ± 5% and 101% ± 9% of the initial K^+ response, respectively. The pD_2 values were 9.96 ± 0.30 and 6.28 ± 0.25, respectively.

In the stem villous arteries U46619 and $PGF_{2\alpha}$ induced concentration-related responses (Fig. 2). In these vessels U46619 produced sigmoid concentration-response curves parallel to the concentration-response curve of $PGF_{2\alpha}$. E_{max} values for U46619 and $PGF_{2\alpha}$ responses were 145% ± 9% and 139% ± 4.4% of the initial K^+ contraction, respectively. The pD_2 values were 8.53 ± 0.01 and 5.93 ± 0.09, corresponding to EC_{50} values of 2.9×10^{-9} and 1.2×10^{-6} mol/L, respectively. As previously shown norepinephine and vasopressin did not induce contractile responses in the fetal stem villous arteries.[14, 15]

Effects of Mg^{++} and isradipine on U46619-induced contractions. Responses to U46619 10^{-7} mol/L were close to maximum (Fig. 1) in intramyometrial preparations. The responses consisted of an initial fast rise in tension, which continued into a maintained contraction with minor fluctuations in tension (Fig. 3). Tension produced by lower concentrations of the compound was characterized by major fluctuations. In the stem villous vessels U46619 10^{-8} mol/L induced a near-maximum response amounting to 120% ± 5% of the initial K^+ contraction (Fig. 2).

At prolonged exposure to U46619 the tension induced was inconstant. To evaluate the effects of Mg^{++} and isradipine on U46619-induced tension the prep-

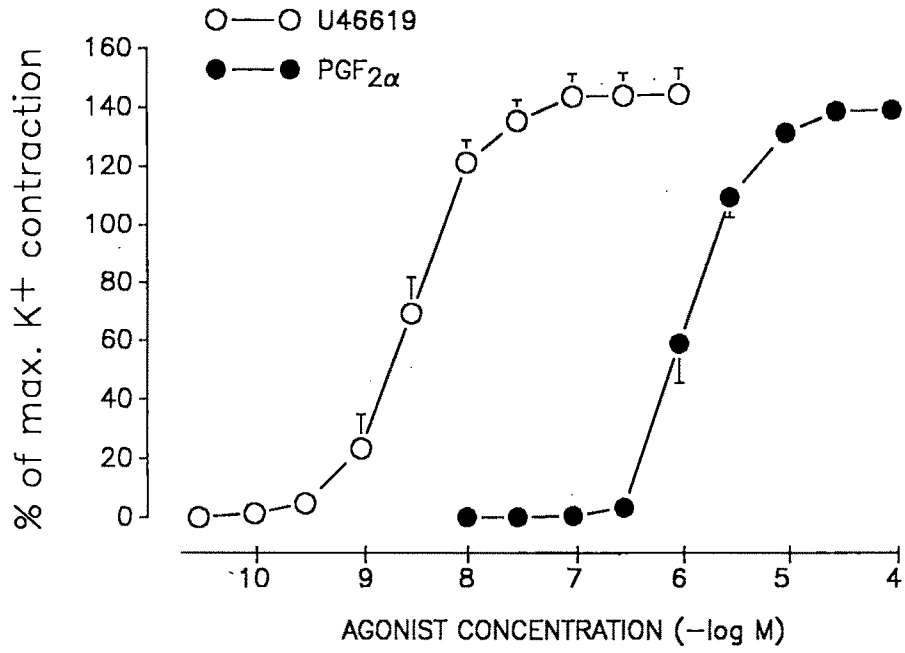


Fig. 2. Fetal stem villous arteries. Concentration-response relations for U46619 10^{-10} to 10^{-6} mol/L and $PGF_{2\alpha}$ 10^{-8} to 10^{-4} mol/L. Each point represents the mean of eight experiments. *Vertical bars* denote SE, when this exceeded the size of the symbols.

arations were exposed to Mg^{++} 0.5 mmol/L and precontracted by U46619 10^{-7} mol/L (maternal vessels) or 10^{-8} mol/L (fetal vessels). The relaxant effects of Mg^{++} or isradipine were then tested by addition of single concentrations of these agents in separate preparations. In maternal preparations Mg^{++} 1.0 to 6.0 mmol/L produced concentration-dependent relaxation of tension induced by U46619 10^{-7} mol/L by up to 43%, and significant relaxation as related to control preparations was obtained at a Mg^{++} concentration of 1.0 mmol/L. In the fetal arteries the relaxant properties of Mg^{++} on U46619-induced contractions were less pronounced than in maternal vessels and significant relaxation appeared at a Mg^{++} concentration of 1.5 mmol/L (Fig. 4, *A*).

Isradipine produced similar concentration-dependent relaxation of U46619-induced tension by up to 33% in both maternal and fetal vessels. Significant relaxation as related to control preparations was obtained after addition of isradipine 10^{-10} mol/L (Fig. 4, *B*). In preparations precontracted by U46619 10^{-7} or 10^{-8} mol/L and exposed to isradipine 10^{-6} mol/L, addition of Mg^{++} 6.0 mmol/L consistently produced further relaxation (Fig. 3).

Effects of endothelial disruption, indomethacin, and digoxin on responses to Mg^{++} and isradipine. Removal of the endothelium or pretreatment with indomethacin 10^{-6} mol/L or digoxin 10^{-6} mol/L did not influence the inhibition produced by Mg^{++} or isradipine on tension produced by U46619 10^{-7} mol/L in maternal intramyometrial arteries or 10^{-8} mol/L in the fetal stem villous arteries.

Effects of Mg^{++} and isradipine on responses to Ca^{++}. The vessels were exposed to Ca^{++}-free medium (EGTA 0.01 mmol/L) for 30 minutes (repeated washing during the initial 15 minutes), and responses to K^+ 124 mmol/L or U46619 (10^{-7} mol/L in maternal vessels and 10^{-8} mol/L in fetal vessels) were elicited after preincubation for 20 minutes with Mg^{++} 6.0 mmol/L, with isradipine 10^{-6} mol/L (Mg^{++} 0.5 mmol/L) or in controls (Mg^{++} 0.5 mmol/L). The development of constant tension was awaited and Ca^{++} was then added cumulatively.

Responses in Ca^{++}-free medium. In Ca^{++}-free medium (EGTA 0.01 mmol/L) responses to K^+ 124 mmol/L were abolished in both types of vessel (Fig. 5, *A* and *B*).

Responses to U46619 (10^{-7} mol/L) in Ca^{++}-free medium in maternal vessels consisted of an initial tension peak, which reached 56% ± 9% (n = 8) of the initial K^+ response. These continued into a lower tension level of about 44% ± 8% (n = 8). In vessels pretreated with Mg^{++} 6.0 mmol/L the response to U46619 was reduced to 45% and 18%, respectively. Isradipine reduced

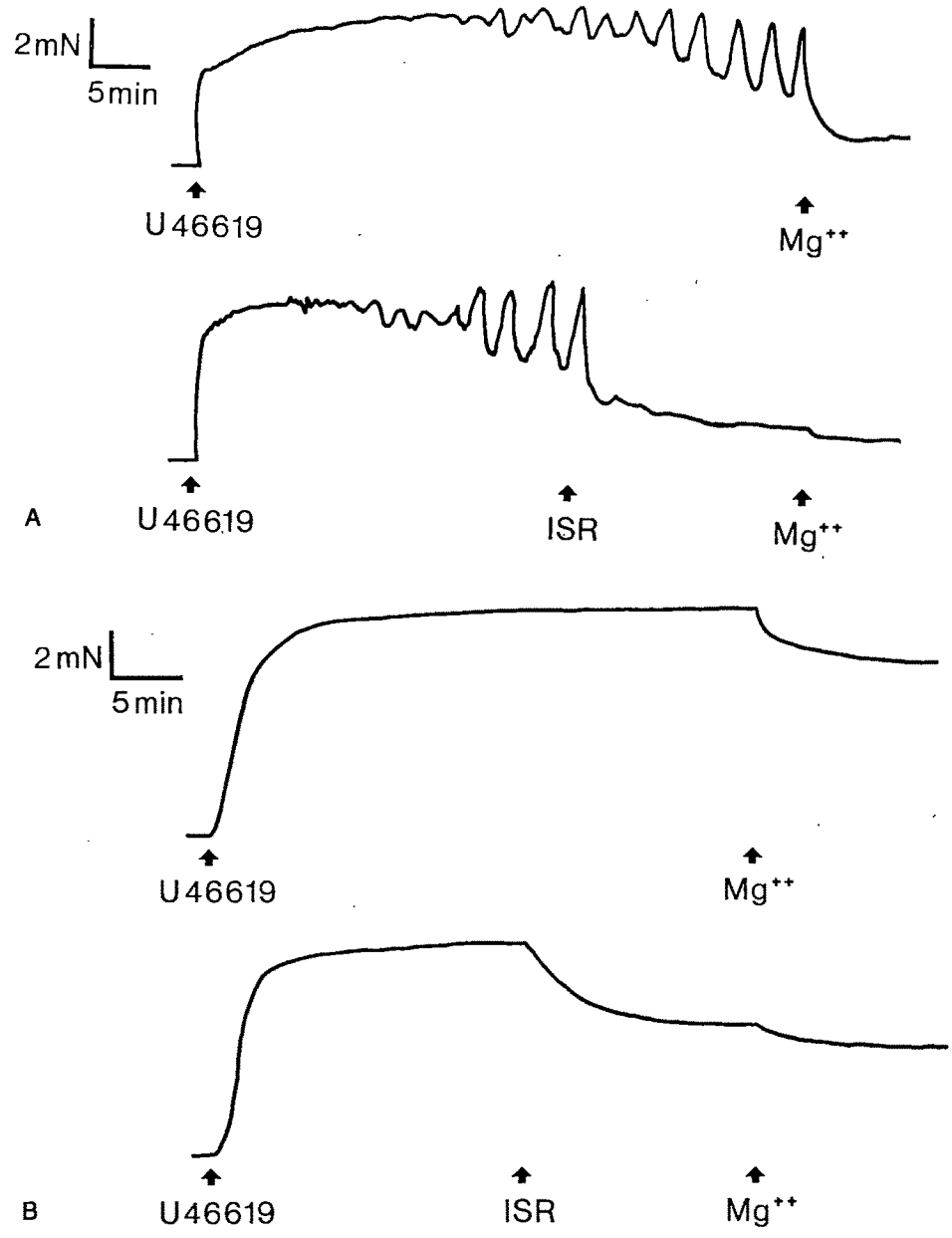


Fig. 3. Maternal intramyometrial arteries **(A)** and fetal stem villous arteries **(B)**: Response to U46619 10^{-7} mol/L (maternal vessels) and 10^{-8} mol/L (fetal vessels) and effects of isradipine *(ISR)* 10^{-6} mol/L and Mg^{++} 6.0 mmol/L.

the response to 40% and 16%, respectively (Fig. 6, *A*).

In fetal vessels U46619 (10^{-8} mol/L) produced a fast rise in tension, which was maintained until the subsequent Ca^{++} addition. The response in Ca^{++}-free medium was reduced from 140% to 35% (n = 8) of the initial K^+ response as compared with the response in normal Krebs solution. Pretreatment with Mg^{++} or isradipine did not cause any further reduction of the U46619 response (Fig. 6, *B*).

Responses to Ca^{++} after K^+ stimulation. The responses produced by addition of Ca^{++} (0.01 to 4.0 mmol/L) after initial stimulation with K^+ (124 mmol/L) in Ca^{++}-free medium were partially depressed by Mg^{++} 6.0 mmol/L. In the maternal intramyometrial arteries the E_{max} and pD_2 values were significantly reduced (Fig. 5, *A*). In stem villous arteries the pD_2 and E_{max} values could not be calculated, since no definite maximum was achieved at Ca^{++} 4.0

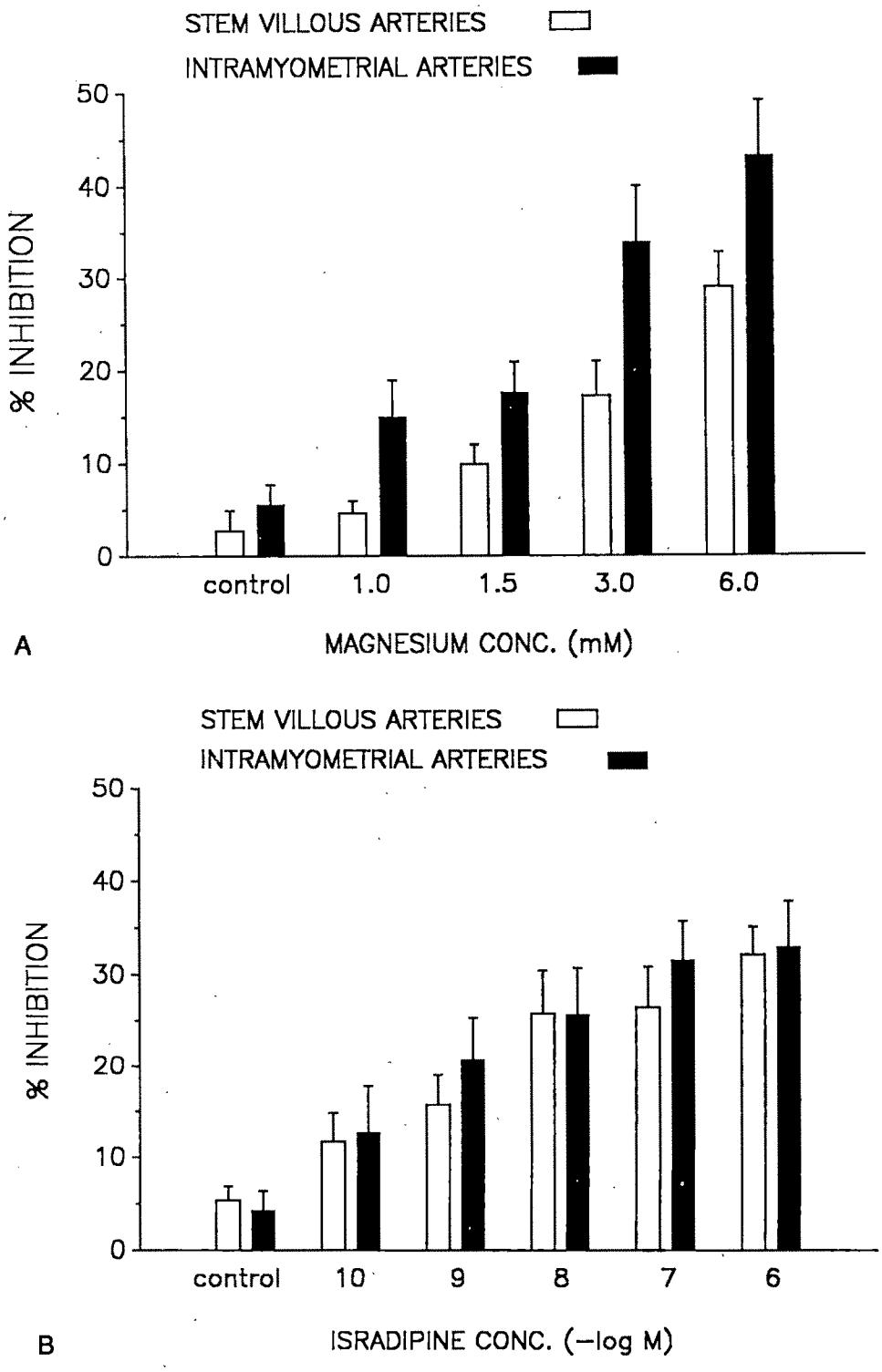


Fig. 4. Maternal intramyometrial arteries and fetal stem villous arteries. Concentration-response relations for Mg^{++} 1.0 to 6.0 mmol/L **(A)** and isradipine 10^{-10} to 10^{-6} mol/L **(B)** in vessels pretreated with U46619 10^{-7} mol/L (maternal vessels) or 10^{-8} mol/L (fetal vessels). Single exposures of Mg^{++} and isradipine in separate preparations were used for these experiments ($n = 8$). *Vertical bars* denote SE.

mmol/L. However, the tension induced by Ca^{++} 0.1 to 0.5 mmol/L was reduced by Mg^{++} as compared with the responses in the control preparation (Fig. 5, *B*), while responses to Ca^{++} 1.0 to 4.0 mmol/L showed no significant decrease.

In both maternal and fetal vessels pretreated with isradipine 10^{-6} mol/L the responses to readdition of Ca^{++} (0.01 to 4. mmol/L) were virtually abolished (Fig. 5, *A* and *B*).

Responses to Ca^{++} after U46619 stimulation. In maternal preparations Mg^{++} 6.0 mmol/L produced partial inhibition of the response to subsequent addition of Ca^{++} (0.01 to 4.0 mmol/L), as compared with preparations exposed to Mg^{++} 0.5 mmol/L. In vessels pretreated with isradipine 10^{-6} mol/L (Mg^{++} 0.5 mmol/L) responses to Ca^{++} (0.01 to 4.0 mmol/L) were even more reduced (Fig. 6, *A*). However, the inhibition of responses to Ca^{++} produced by isradipine 10^{-6} mol/L and Mg^{++} 6.0 mmol/L did not differ significantly.

In fetal stem villous arteries Mg^{++} (6.0 mmol/L) and isradipine (10^{-6} mol/L) produced a similar inhibition of the responses to Ca^{++} 0.01 to 4.0 mmol/L (Fig. 6, *B*).

Comment

The TxA_2 analog U46619 is a potent vasoconstrictor of umbilical and placental vessels as shown in vitro on human preparations[5, 16, 17] and in animal in vivo experiments.[3] In accordance with previous studies the present results showed that U46619 produced concentration-dependent vasoconstriction in isolated human uteroplacental arteries. Lower levels of U46619 were needed to produce significant contraction of maternal arteries as compared with fetal arteries. Furthermore, the concentration-response curves showed different profiles in maternal and fetal vessels showing a biphasic course in the intramyometrial arteries. Similar pD_2 values were assessed for fetal vessels and for the late part of the concentration-response curve in the maternal vessels, while a significantly higher pD_2 value was estimated for the first part of the concentration-response curve in the maternal vessels. It cannot be excluded that these results reflect the existence of two subtypes of TxA_2 receptors in the maternal intramyometrial vessels. Further studies including specific receptor antagonists are needed to clarify this possibility.

In preeclampsia there seems to be an imbalance in the synthesis ratio between prostacyclin and TXA_2, TXA_2 being produced in excess.[4] However, it has been shown that preeclampsia is not accompanied by changes in TXA_2 production that can be detected in maternal blood.[18] On the other hand, an increased production of TXA_2 was found in placentas from hypertensive pregnancies,[19] suggesting that a local disturbance in TXA_2/PGI_2 balance could be responsible for the increased resistance in the uteroplacental vascular bed. Together with those of others, our results strengthen the hypothesis that TXA_2 is partly responsible for the vasospasm in uteroplacental vessels, which is a constant feature in the syndrome of preeclampsia.[2]

The effects of Mg^{++} on the uteroplacental resistance vessels have recently been evaluated,[8] and it has been demonstrated that Mg^{++} relaxes vessels, which is important for resistance regulation in the human uteroplacental vascular bed. In this in vitro study Mg^{++} in concentrations that are readily reached during therapeutic use[20] effectively relaxed U46619-induced contractions as compared with responses in control preparations exposed to 0.5 mmol/L Mg^{++}. In late pregnancy the concentrations of Mg^{++} in plasma range from 0.5 to 0.8 mmol/L (median about 0.65 mmol/L, $n = 2000$) (Skajaa K. Unpublished observations), with subsequent free extracellular Mg^{++} of about 0.5 mmol/L.

Mg^{++} seems to inhibit transmembrane calcium influx,[6, 8] but several intracellular mechanisms involved in the activation of vascular contraction may be influenced by the ion.[6, 21-23] These results give further support to the view that Mg^{++} inhibits Ca^{++} influx through voltage-operated channels, since the K^+-induced contractions were reduced. Responses to U46619 and other prostanoids seem to be mediated by an increase in intracellular Ca^{++} brought about partly by a transmembrane Ca influx via receptor-operated channels and partly by a release of Ca from intracellular stores.[24] The response to U46619 in this study and to $PGF_{2\alpha}$ as shown previously[8] were partly inhibited by Mg^{++}, as were the responses to Ca^{++} after stimulation with U46619 in calcium-free medium. Thus Mg^{++} may interfere with Ca^{++} entry through receptor-operated channels, as well as with Ca^{++} release from intracellular stores.[21] However, the exact mechanisms by which Mg^{++} exerts its inhibitory effects are still unclear.

Calcium antagonists of the dihydropyridine type have a common action to inhibit Ca^{++} influx into smooth muscle cells and subsequent contraction. The dihydropyridine receptor is a component of the voltage-dependent calcium channels,[25, 26] which explains the effective blockade of Ca^{++} entry through these channels by the dihydropyridine calcium antagonists. Although this is the primary site of action of calcium antagonist, some other sites of action cannot be excluded. In this study it clearly emerged that isradipine very effectively hindered the Ca^{++} influx through voltage-operated Ca channels as the response

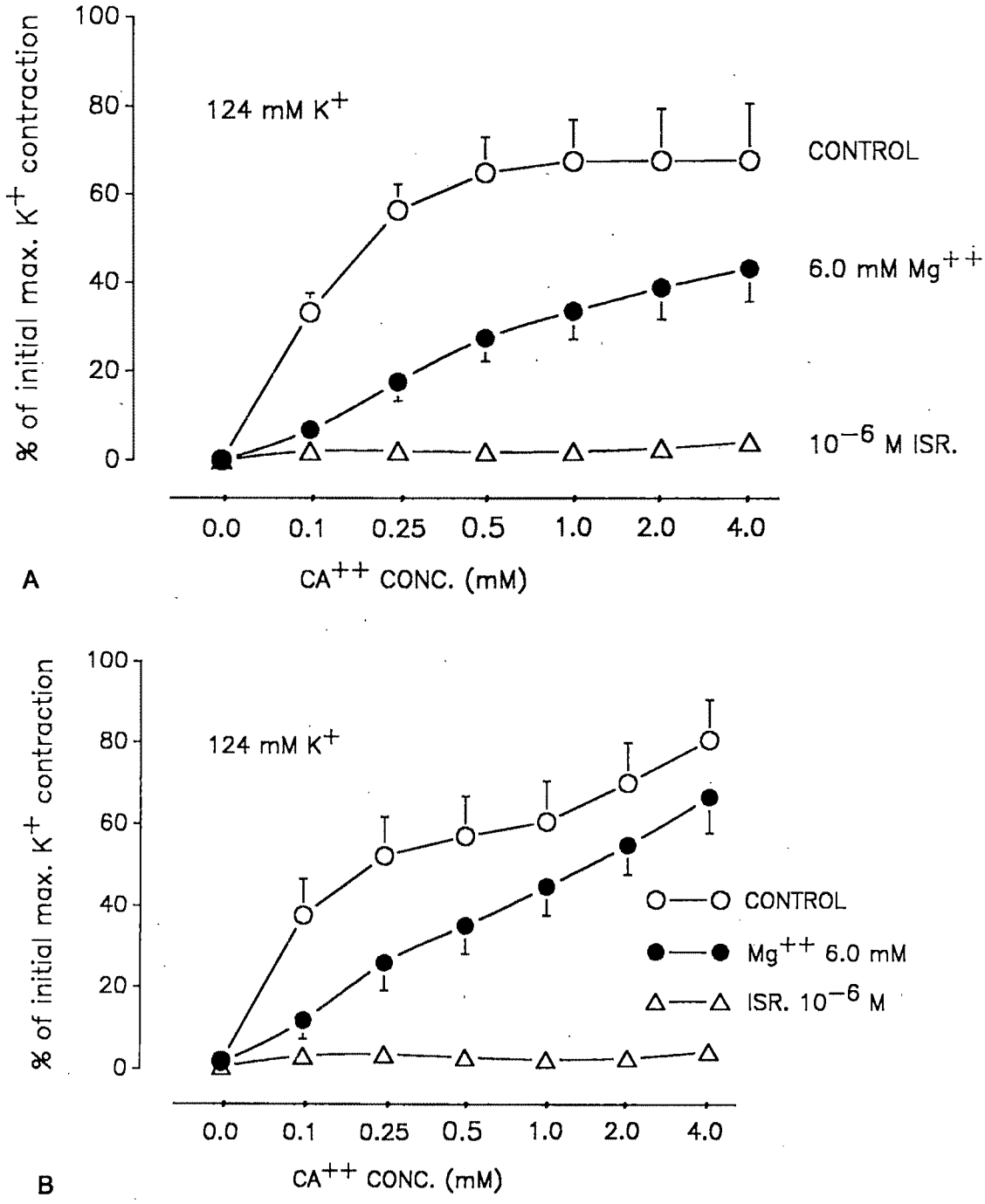


Fig. 5. Maternal intramyometrial arteries (**A**) and fetal stem villous arteries (**B**) preincubated in Ca^{++}-free medium. Concentration-response relations for Ca^{++} 0.1 to 4.0 mmol/L in vessels exposed to isradipine *(ISR)*. 10^{-6} mol/L or Mg^{++} 6.0 mmol/L after initial depolarization with K^+ 124 mmol/L. Each point represents the mean of eight experiments. *Vertical bars* denote SE, when this exceeded the size of the symbols.

to high K^+ concentration was completely abolished. However, the fact that the U46619 response in Ca^{++}-free medium was reduced by isradipine suggests other actions of isradipine as well.

In this study the effects of Mg^{++} and isradipine were similar although isradipine was more effective with respect to inhibition of Ca^{++} influx as reflected by effects on K^+-induced contractile activation. However, Mg^{++} induced a further relaxation in preparations precontracted with U46619 and then exposed to isradipine 10^{-6} mol/L, i.e., producing maximum (maternal vessels) or near-maximum (fetal vessels) inhibition. This could reflect that Mg^{++} interfered with membrane and/or other cellular mechanisms[21, 22] that are unaf-

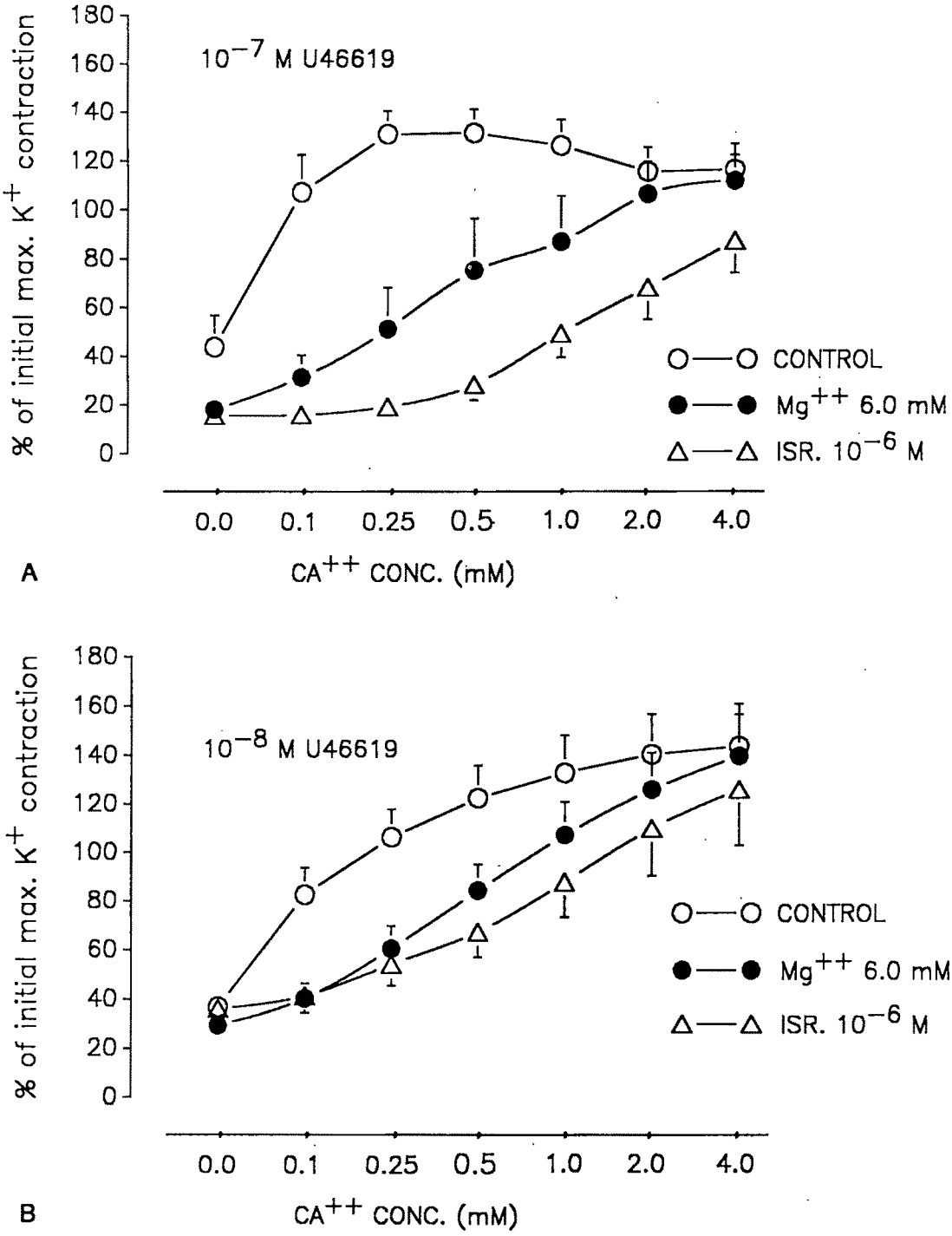


Fig. 6. Maternal intramyometrial arteries (**A**) and fetal stem villous arteries (**B**) preincubated in Ca^{++}-free medium. Concentration-response relations for Ca^{++} 0.1 to 4.0 mmol/L in vessels exposed to isradipine *(ISR)*. 10^{-6} mol/L or Mg^{++} 6.0 mmol/L after initial stimulation with U46619 10^{-7} mol/L (maternal vessels) or 10^{-8} mol/L (fetal vessels). In the maternal vessels the response to U46619 in Ca-free medium consisted of an initial peak, which continued into a lower tension level (see text). Only the lower tension level of the response is shown in the figure. Each point represents the mean of eight experiments. *Vertical bars* denote SE, when this exceeded the size of the symbols.

fected by dihydropyridine calcium antagonists such as isradipine. However, other explanations are possible. Thus isradipine may have changed the calcium channel sensitivity to Mg^{++}.

If treatment of preeclampsia is required, improvement of uterine and placental blood flow is crucial, particularly as the systemic hypertension may be considered a consequence rather than the cause of the

disease.[2] The relation between fetal and maternal uteroplacental vascular effects of Mg^{++} is therefore of considerable clinical interest. Apparently, the effects of increasing concentrations of Mg^{++} include relaxation of both fetal and maternal uteroplacental arteries, but the effects were more pronounced in the maternal vessels. In addition Mg^{++} produces relaxation of the myometrium,[27, 28] and, together with the preference of the maternal vasculature, this could be of advantage in the treatment of preeclampsia. However, the hemodynamic effects of such treatment depend on the overall systemic response. In the pregnant rabbit Mg^{++} infusion increased uterine blood flow.[29] Similar studies on uteroplacental blood flow in human pregnancies are sparse, but Mg^{++} infusion in human volunteers has been shown to reduce the systemic blood pressure concomitant with a significant increase in renal blood flow and in excretion of prostacyclin metabolites.[30] Moreover, Mg^{++} in therapeutic concentrations mediates enhanced production of prostacyclin by cultured human umbilical vein endothelial cells.[9] Thus it seems that Mg^{++} in vivo may alleviate the action of TxA_2 in several respects. However, enhanced prostacyclin production does not seem of importance for the relaxant responses to Mg^{++} in isolated human uteroplacental arteries since no effects of indomethacin or of endothelial removal were found. The relaxant effects of Mg^{++} did not seem to be mediated by a stimulation of the sodium-potassium-adenosine triphosphatase, since pretreatment with digoxin, which inhibits this enzyme, did not influence the action of Mg^{++} on U46619-induced contractions.

In conclusion, our results show that Mg^{++} and the calcium antagonist isradipine inhibit contraction produced by TxA_2 in uteroplacental resistance vessels. If preeclampsia-eclampsia involves increased vascular actions of TxA_2, these data give support to the established use of magnesium sulfate in the treatment of these conditions and suggest that calcium antagonists may be of potential benefit.

We thank Ms. Kirsten Aggerholm for skilled technical assistance.

REFERENCES

1. Lunell N-O, Nylund LE, Lewander R, Sarby B. Uteroplacental blood flow in preeclampsia. Measurements with indium 113 m and a computer-link gamma camera. Clin Exp Hypertens 1982;B1:105-17.
2. Romero R, Lockwood C, Oyarzun E, Hobbins JC. Toxemia: new concepts in an old disease. Semin Perinatol 1988;12:302-23.
3. Parisi VM, Walsh SW. Fetoplacental vascular responses to prostacyclin after thromboxane-induced vasoconstriction. Am J Obstet Gynecol 1989;160:502-7.
4. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol 1985;152:335-40.
5. Glance DG, Elder MG, Myatt L. The actions of prostaglandins and their interactions with angiotensin II in the isolated perfused human placental cotyledon. Br J Obstet Gynaecol 1986;93:488-94.
6. Altura BM, Altura BT, Carella A, Gebrewold A, Murakawa T, Nishio A. Mg^{++}-Ca^{++} interaction in contractility of vascular smooth muscle: Mg^{++} versus organic calcium channel blockers on myogenic tone and agonist-induced responsiveness of blood vessels. Can J Physiol Pharmacol 1987;65:729-45.
7. Altura BM, Altura BT, Carella A. Magnesium deficiency-induced spasms of umbilical vessels: relation to preeclampsia, hypertension, growth retardation. Science 1983;221:376-8.
8. Skajaa K, Forman A, Andersson KE. Effects of magnesium on isolated human fetal and maternal vessels. Acta Physiol Scand 1990;139:551-9.
9. Watson KV, Moldow CF, Ogburn PL, Jacob HS. Magnesium sulfate: rationale for its use in preeclampsia. Proc Natl Acad Sci USA 1986;83:1075-8.
10. Walters BNJ, Redmann CWG. Treatment of severe pregnancy-associated hypertension with the calcium antagonist nifedipine. Br J Obstet Gynaecol 1984;91:330-6.
11. Allen J, Maigaard S, Forman A, et al. Acute effects of nitrendipine in pregnancy induced hypertension. Br J Obstet Gynaecol 1987;94:222-6.
12. Maigaard S, Forman A, Brogaard-Hansen KP, Andersson KE. Inhibitory effects of nitrendipine on myometrial and vascular smooth muscle in human pregnant uterus and placenta. Acta Pharmacol Toxicol 1986;59:1-10.
13. Högestätt ED, Andersson K-E, Edvinsson L. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. Acta Physiol Scand 1983;117:49-61.
14. Maigaard S, Forman A, Andersson KE. Differential effects of angiotensin, vasopressin and oxytocin on various smooth muscle tissue within the human uteroplacental unit. Acta Physiol Scand 1986;128:23-32.
15. Maigaard S, Forman A, Andersson KE. Relaxant and contractant effects of some amines and prostanoids in myometrial and vascular smooth muscle within the human uteroplacental unit. Acta Physiol Scand 1986;128:33-44.
16. Mak KK-W, Gude NM, Walters WAW, Boura ALA. Effects of vasoactive autocoids on the human umbilical-fetal placental vasculature. Br J Obstet Gynaecol 1984;91:99-106.
17. Bjøro K, Stray-Pedersen S. Effects of vasoactive autocoids on different segments of human umbilical-placental vessels. Gynecol Obstet Invest 1986;22:1-6.
18. Ylikorkala O, Jouppila P, Kirkenen P, Viinika L. Maternal thromboxane, prostacyclin and umbilical blood flow in humans. Obstet Gynecol 1984;63:677-80.
19. Mäkilä UM, Viinika L, Ylikorkala O. Increased thromboxane A_2-production but normal prostacyclin by the placenta in hypertensive pregnancy. Prostaglandin 1984;27:87-95.
20. Sibai BM, Graham JM, McCubbin JH. A comparison of intravenous and intramuscular magnesium sulfate regimens in preeclampsia. Am J Obstet Gynecol 1984;150:728-33.
21. Saida K. Intracellular Ca release in skinned smooth muscle. J Gen Physiol 1982;80:191-202.
22. Karaki H, Hatano K, Weiss GB. Effects of magnesium on ^{45}Ca uptake and release at different sites in rabbit aortic smooth muscle. Pflugers Arch 1983;398:27-32.
23. Levine BS, Coburn JW. Magnesium, the mimic/antagonist of calcium [Editorial]. N Engl J Med 1984;310:1253-5.
24. Uski TK, Andersson K-E. Effects of prostanoids on isolated feline cerebral arteries. II. Roles of extra- and intracellular calcium for $PGF_{2\alpha}$ induced contraction. Acta Physiol Scand 1984;120:197-205.
25. Nayler WG. Calcium antagonists. London: Academic Press, 1988:87-100.
26. Skattebøl A, Brown AM, Triggle DJ. Homologous regu-

lation of voltage-dependent calcium channels by 1,4-dihydropyridines. Biochem Biophys Res Commun 1989; 160:929-36.
27. Hall DG, McGaughey HS, Corey EL, Thornton WN. The effects of magnesium therapy on duration of labor. Am J Obstet Gynecol 1959;78:27-32.
28. Spisso KR, Harbert GM Jr, Thiagarajah S. The use of magnesium sulfate as the primary tocolytic agent to prevent premature delivery. Am J Obstet Gynecol 1982; 142:840-5.
29. Ferris TF, Weir EK. Effect of captopril on uterine blood flow and prostaglandin E synthesis in pregnant rabbit. J Clin Invest 1983;71:809-15.
30. Nadler JL, Goodson S, Rude R. Evidence that prostacyclin mediates the vascular action of magnesium in humans. Hypertension 1987;9:379-83.

The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes

George Lewandowski, MD, Gregorio Delgado, MD, Robert W. Holloway, MD, Michael Farrell, PhD, A. Bennett Jenson, MD, and Wayne D. Lancaster, PhD
Washington, D.C.

Southern blot hybridization has been used to identify human papillomavirus types in both primary tumors and lymph node metastases. However, this technique requires fresh-frozen tissue and is incapable of localizing deoxyribonucleic acid sequences to specific cell types in the tumor sample. In contrast, in situ hybridization precisely locates viral sequences within tumor cells while preserving cellular architecture. Further, in situ hybridization requires only small samples of formalin-fixed, paraffin-embedded tissues. Five lymph nodes (from four patients) containing metastatic cervical squamous tumor cells (identified with hematoxylin and eosinophil staining) were analyzed with in situ hybridization techniques with human papillomavirus type 16 deoxyribonucleic acid probes labeled with sulfur 35. The primary cervical cancer from all four patients had been shown to contain human papillomavirus type 16 sequences by Southern blot. Three specimens from two patients clearly showed the presence of human papillomavirus type 16 sequences within the nuclei of metastatic tumor cells, whereas two specimens were nondiagnostic most likely as a result of the small volume of cancer relative to the size of the lymph node. This information indicates that it is the tumor cells themselves that contain viral deoxyribonucleic acid and provides additional evidence linking human papillomavirus with cervical carcinogenesis. (Am J Obstet Gynecol 1990;163:1333-7.)

Key words: Human papillomavirus infection, metastatic cervical cancer, in situ hybridization

Preinvasive and invasive lower genital neoplasms have been broadly linked to a sexually transmissable agent with a number of etiologic factors presented by LaVecchia et al.[1] Early work by Meisels et al.[2] showed koilocytic atypia, which is pathognomonic for human papillomavirus (HPV) infection, in 70% of patients with cervical dysplasia. Kurman et al.,[3] Syrjanen,[4] Walker et al.,[5] and others used the immunoperoxidase technique to show specific papillomavirus antigens in cervical and vulvar lesions, but found that lesions which were less well differentiated did not retain the capability to synthesize capsid proteins into virions and therefore were not detectable by this technique. An additional drawback to our understanding of the relationship between HPV infection and oncogenesis has been the inability to grow HPV in cell culture. As a result, further definition of the role of HPV infection in carcinogenesis has relied on refinements in the techniques of molecular biology.

Southern blot hybridization[6] tissue typing of fresh or frozen tissue has been the "gold standard" method of

From the Division of Gynecologic Oncology and the Department of Pathology, Georgetown University School of Medicine.
Supported in part by Public Health Service Grants CA 32603 and CA 32638.
Received for publication December 21, 1989; revised May 24, 1990; accepted June 15, 1990.
Reprint requests: George Lewandowski, MD, Division of Gynecologic Oncology, Ohio State University Hospitals, Fifth Floor, Means Hall, 1654 Upham Drive, Columbus, OH 43210.
6/1/23228

HPV detection, and has been used in the majority of reports dealing with HPV-related disease. A number of prospective studies[7-11] showed that HPV types 6 and 11 carry a low risk for progression to dysplasia and neoplasia, whereas HPV types 16, 18, 31, 33, and 35 are associated with many invasive lesions and seem to constitute a higher risk group.

Lancaster et al.[12] and Fuchs et al.[13] provided novel evidence in support of this association with the demonstration of the same HPV type in both primary tumors and their lymph node metastases, thus further implicating HPV in carcinogenesis. However, because this work was performed with the Southern blot hybridization technique, it was impossible to show with certainty whether the HPV deoxyribonucleic acid (DNA) was within actual tumor cells, or whether it merely existed within adjacent lymphocytes or lymphatic stroma.

To address this issue, in situ hybridization was developed to provide HPV typing information while preserving cellular architecture. The specific goal of this work was to show the presence of HPV type 16 DNA sequences in tumor cells within metastatic lymph nodes.

Material and methods

Source and handling of tissue. Tissues obtained from lymph node specimens of patients undergoing surgery for cervical malignancies at Georgetown University Hospital were analyzed with the use of frozen section. If tumor involvement was shown, a segment of the lymph node was obtained, frozen on dry ice, and stored at −70° C. The corresponding paraffin blocks that contained tumor-bearing lymphatic tissue were obtained from the Department of Pathology in each case in which frozen tissue was secured.

Southern blot hybridization. The frozen specimens described above were analyzed for the presence of HPV types 6/11, 16, and 18 by Southern blot hybridization.[6] HPV DNA probes radiolabeled with phosphorus 32 were prepared by nick translation with *Escherichia coli* DNA polymerase I. Blot transfer and hybridization were performed under moderately stringent conditions detailed elsewhere.[13]

In situ hybridization. Histologic analysis was done to confirm the presence of tumor in the paraffin-embedded lymph node section obtained. Adjacent serial sections 4 to 6 μm thick were mounted on slides that had been acid-cleaned and treated with poly-L-lysine solution.

HPV 6 and HPV 16 genomes were isolated from molecular clones described previously.[14] Viral DNA was released from the pBR322 vector by digestion with Bam HI and isolated by agarose gel electrophoresis and electroelution.

The HPV types 6 and 16 DNA fragments obtained were radiolabeled with ^{35}S deoxyadenosine triphosphate (New England Nuclear, Boston) with the "oligolabeling" technique of Feinberg and Vogelstein.[15] The specific activity of the probes determined with scintillation counting was found to range from 1.8 to 2.1×10^7 counts per minute per microgram of DNA. Radioactive probe fragments were separated from unincorporated nucleotide with a commercially available exchange resin (Nensorb, New England Nuclear) and concentrated with ethanol precipitation.

In situ hybridization analyses were performed on the tumor metastases as described by Brahic and Haase[16] and Gupta et al.[17] with the following modifications. After removal of paraffin and the initial dehydration, the tissue sections were incubated in 1 μg/ml proteinase K in phosphate-buffered saline solution for 20 minutes. With 25 to 30 μl of hybridization mixture (50% deionized formamide, 10% dextran sulfate, 0.6 mol/L sodium chloride, 0.01 mol/L Tris hydrochloride (pH 7.4), 0.5 mmol/L ethylenediaminetetraacetic acid, 1 mg/ml bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.2 mg/ml salmon sperm DNA, 0.5 mM dithiothreitol and 0.2 μg/ml of HPV type 6 or 16 DNA probes labeled with ^{35}S, hybridizations were carried out for 50 to 60 hours at 37° C. After the hybridizations, slides were washed and dehydrated by the method described by Gupta et al.[17]

Autoradiography. After air drying, the slides were dipped in photographic emulsion (nuclear tract NTB-3, Kodak, Rochester, N.Y.) which had been melted and maintained at 45° C. Exposure was performed in desiccated, light-free containers for 5 to 7 days at 4° C. The emulsion was developed following manufacturer's recommendations, and slides were stained with hematoxylin before the coverslips were positioned.

Results

Five different lymph nodes containing metastatic cervical squamous cancer from four patients were analyzed. Two separate nodal specimens were studied from one patient, representing metastases from paraaortic and scalene lymph node biopsy specimens obtained 9 months apart. The primary tumor from each of the four patients had been found to contain HPV type 16 with the use of Southern blot hybridization. The Southern blot demonstration of HPV type 16 involvement in these cases has already been reported.[12]

Three nodal samples from two patients showed the presence of HPV type 16 DNA within nests of tumor cells in the lymph nodes. In no instance did the control HPV type 6 DNA hybridize to the samples. Fig. 1, *A* and 1, *B*, show low- and high-power magnifications of one of these specimens. The darkened areas of silver granule deposition indicate probe hybridization within the tumor metastases but not in the surrounding lym-

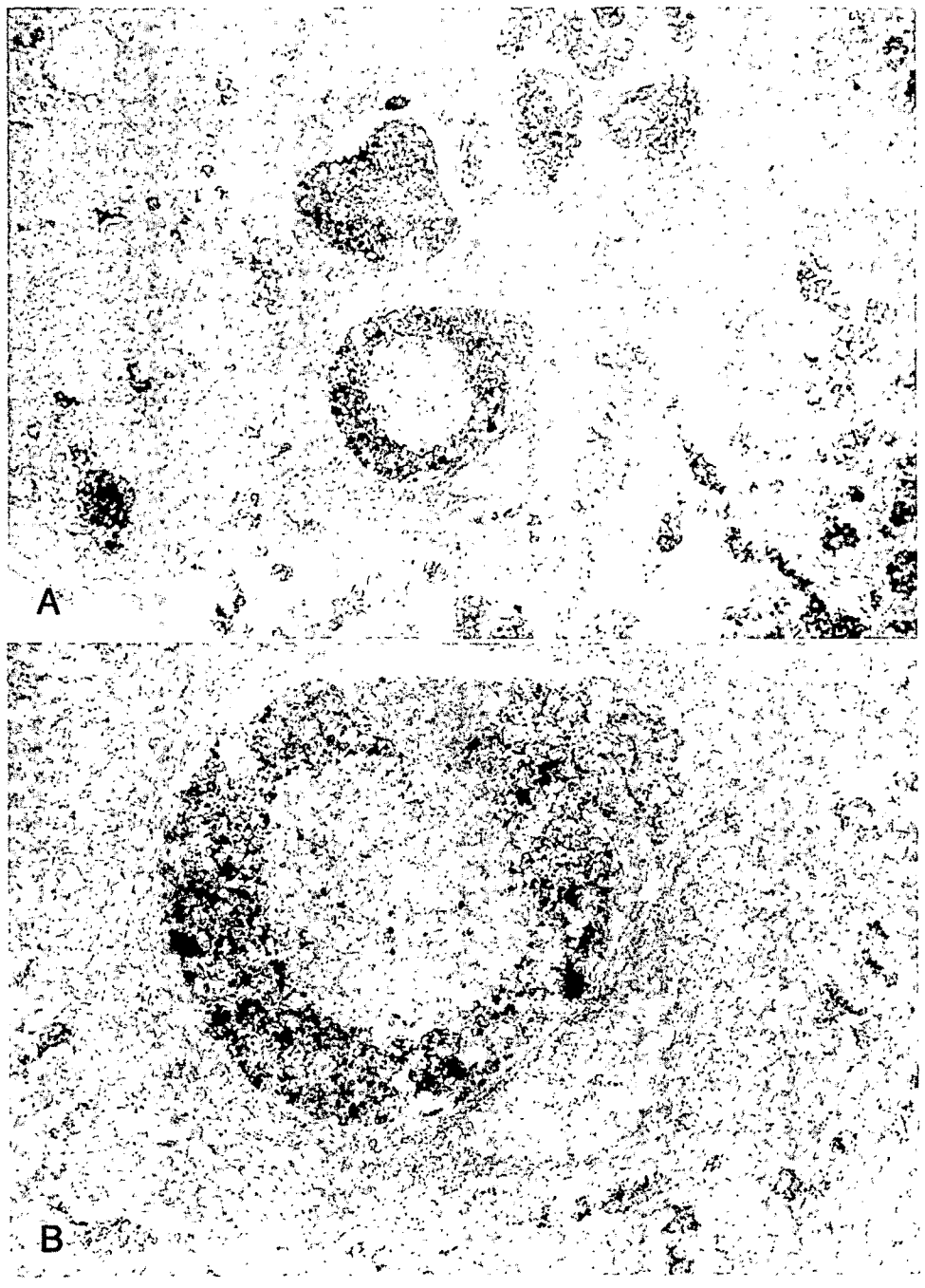


Fig. 1. In situ hybridization analysis with HPV type 16 DNA probe labeled with ^{35}S. **A,** Low-power magnification shows a concentration of black granules indicating positive reaction within tumor metastases. **B,** Note weak hybridization within central necrosis in high-power magnification.

phocytes or stroma. In the high-power magnification view (Fig. 1, *B*) a central area of necrosis within the metastatic nest shows low hybridization intensity corresponding to an area of nonviable cells that presumably contain degraded DNA. Assays of lymph nodes from the remaining two patients were suggestive of HPV type 16 involvement, showing weak probe hybridization. Unfortunately, the microscopic nature of tumor foci within these nodes and tissue loss from the slides kept us from declaring these specimens to be definitively positive for HPV type 16.

Comment

We have used in situ molecular hybridization to show the presence of HPV type 16 DNA within tumor metastases to lymph nodes. In situ hybridization largely

preserves cellular architecture. This allowed us to show that the viral genomes were within the tumor cells and not in the surrounding lymphocytes. Further, this evidence of the virus was found at sites distant from and noncontiguous to the primary lesion, the presumed site of viral entry into the system. This information answers an objection to our earlier use of Southern blot hybridization that showed HPV types in metastatic lymph nodes. It further pinpoints the HPV DNA to the tumor cells themselves.

Walboomers et al.[18] reported a single case in which in situ hybridization was used to indicate the presence of HPV type 16 in a fresh-frozen pelvic tumor metastasis from an endocervical adenocarcinoma. They found that the in situ hybridization positivity was roughly correlated to the copy number of the DNA within the metastasis. Nuovo and Richart[19] corroborate this information in their comparison of slot blot, Southern blot, and in situ hybridization, and added that cases with weak hybridization signals generally reflected less dysplastic lesions. Our demonstration of strong signals in hybridizations from separate lymph node tumor metastases suggests a possible clinical role for in situ hybridization that may ultimately offer a clue as to HPV-related tumor biology and prognosis.

In situ hybridization offers an additional advantage of using formalin-fixed, paraffin-embedded tissues for analysis. Duplicate examinations are therefore possible, which will allow for further testing should assay modifications or new HPV types be discovered. Only a single report has used Southern blotting on paraffin-embedded tissue,[20] and the technique requires special tissue handling that may not be applicable to standard surgical pathologic procedures.

However, in situ hybridization is not without drawbacks. We found in situ hybridization to be technically more difficult and as a result potentially less sensitive than Southern blotting. Individual assays may take from 2 weeks to several months depending on the specific activity of the probe and the condition and amount of viral DNA within the specimen. Caussey[21] found a 30% detection rate with in situ hybridization compared with rates between 70% and 89% for cervical cancers with the use of blot technology. Further investigation into HPV-related carcinogenesis will depend on information from both in situ and Southern blot hybridization techniques as well as the development and application of newer techniques including the polymerase chain reaction.

In conclusion, with the use of in situ hybridization to show HPV type 16 DNA in tumor metastases within lymph nodes we have provided additional evidence that implicates HPV infection with lower genital carcinogenesis. In situ hybridization offers the advantages of repeated assay along with its use of formalin-fixed, paraffin-embedded tissues, but seems to suffer from assay sensitivity. Both in situ hybridization and Southern blotting may provide important information on the HPV-related molecular biology of invasive and preinvasive lesions in the lower genital tract.

REFERENCES

1. LaVecchia C, Franceschi S, DeCarli A, et al. Sexual factors, venereal disease, and the risk of intraepithelial neoplasia and invasive cervical neoplasia. Cancer 1986;58:935-41.
2. Meisels A, Fortin M, Roy M. Condylomatous lesions of the cervix. II. Cytologic, colposcopic and histologic study. Acta Cytol 1977;21:379-90.
3. Kurman RJ, Shah KH, Lancaster WD, Jenson AB. Immunoperoxidase localization of papillomavirus antigens in cervical dysplasias and vulvar condylomas. Am J Obstet Gynecol 1981;140:931-5.
4. Syrjanen KF. Current concepts of human papillomavirus infections in the genital tract and their relationship to intraepithelial neoplasia and squamous cell carcinoma. Obstet Gynecol Surv 1984;39:252-65.
5. Walker PG, Singer A, Dyson JL, Shah KV, To H, Coleman DV. The prevalence of human papillomavirus antigen in patients with cervical intraepithelial neoplasia. Br J Cancer 1983;48:99-103.
6. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Bio 1977;113:237-42.
7. DeVilliers EM, Schneider A, Miklaw H, et al. Human papillomavirus infections in women with and without abnormal cervical cytology. Lancet 1987;2:703-5.
8. Campion MJ, Cuzich J, McCance DJ, Singer A. Progressive potential of mild cervical atypia: prospective cytological, colposcopic, and virological study. Lancet 1986; 2:237-40.
9. Syrjanen K, Vayrynen M, Saarikoski S, et al. Natural history of cervical human papillomavirus (HPV) infections based on prospective follow-up. Br J Obstet Gynaecol 1985;92:1086-92.
10. Lorincz AT, Temple GF, Kurman RJ, Jenson AB, Lancaster WD. The oncogenic association of specific HPV types with cervical neoplasia. JNCI 1987;79:124-32.
11. Kochel HG, Teichmann A, Eckhardt N, et al. Occurrence of human papillomavirus DNA types 16 and 18 (HPV 16/18) in cervical smears as compared to cytological findings. Int J Gynaecol Obstet 1990;31:145-52.
12. Lancaster W, Castellano C, Santos C, Delgado G, Kurman RJ, Jenson AB. Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites. Am J Obstet Gynecol 1986;154:115-119.
13. Fuchs PG, Girardi F, Pfister H. Human papillomavirus 16 DNA in cervical cancers and in lymph nodes of cervical cancer patients: a diagnostic marker for early metastases. Int J Cancer 1988;41:41-5.
14. de Villiers EM, Gissmann L, zur Hausen H. Molecular cloning of viral DNA from human genital warts. J Virol 1981;40:932-5.
15. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983;132:6-13.
16. Brahic M, Haase A. Detection of viral sequences of low reiteration frequency by in situ hybridization. Proc Natl Acad Sci USA 1978;75:6125-9.
17. Gupta J, Gendelman HE, Naghashfar Z, et al. Specific identification of human papillomavirus type in cervical

smears and paraffin sections by in situ hybridization with radioactive probes. Int J Gynecol Pathol 1985;4:211-5.
18. Walboomers JMM, Fokke HE, Polak M, et al. In situ localization of human papilloma virus type 16 DNA in a metastasis of an endocervical adenocarcinoma. Intervirology 1987;27:81-5.
19. Nuovo GJ, Richart RM. A comparison of slot blot, Southern blot, and in situ hybridization analyses for human papillomavirus DNA in genital tract lesions. Obstet Gynecol 1989;74:673-8.
20. Dubeau L, Chandler L, Gralow J, Nichols PW, Jones PA. Southern blot analysis of DNA extracted from formalin-fixed pathology specimens. Proc Natl Acad Sci USA 1986;84:2964-67.
21. Caussy L, Orr W, Daya AD, et al. Evaluation of methods for detecting human papillomavirus deoxyribonucleotide sequences in clinical specimens. J Clin Microbiol 1988; 26:236-41.

Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy

Miguel Isla, DVM, PhD, and Donald C. Dyer, PhD
Ames, Iowa

The differential inhibitory effect of the vasodilators on contractile responses to norepinephrine, serotonin, and potassium on isolated uterine artery ring segments from pregnant ewes within 2 weeks of term was quantified and correlated with the source of Ca^{++} for the vasoconstrictors producing the smooth muscle contraction. The contraction evoked by the vasoconstrictors was dependent on extracellular Ca^{++} and in agonist-induced contractions also on an intracellular pool of Ca^{++}. Nifedipine effectively inhibited K^+-induced (90 mmol/L) contractions (antagonist concentration to reduce the maximum contractile effect to the agonist to 50%, $1.95 \pm 0.9 \times 10^{-8}$ mol/L), whereas it was relatively ineffective in blocking norepinephrine-induced (10^{-5} mol/L) or serotonin-induced (10^{-5} mol/L) vasoconstriction (antagonist concentration to reduce the maximum contractile effect to the agonist to 50%, $1.38 \pm 0.4 \times 10^{-4}$ mol/L and $2.04 \pm 0.4 \times 10^{-5}$ mol/L, respectively). Methoxyverapamil (D-600) strongly inhibited serotonin-induced contractions (antagonist concentration to reduce the maximum contractile effect to the agonist to 50%, $3.3 \pm 0.3 \times 10^{-7}$ mol/L). The phasic rather than the tonic components of the serotonin- and norepinephrine-induced contractions were more effectively inhibited by D-600 ($p < 0.05$). Sodium nitroprusside preferentially blocked ($p < 0.05$) the sustained tonic components of norepinephrine- and serotonin-induced vasoconstrictions (antagonist concentration to reduce the maximum contractile effect to the agonist to 50%, $7.1 \pm 0.4 \times 10^{-7}$ mol/L and $8.2 \pm 0.6 \times 10^{-7}$ mol/L, respectively). On the basis of these findings it is concluded that D-600 and sodium nitroprusside are more effective than nifedipine in blocking contractile responses due to receptor stimulation, and therefore might be more effective in the treatment of hypertensive emergencies in which these amines might be implicated. (Am J Obstet Gynecol 1990;163:1337-44.)

Key words: Nifedipine, methoxyverapamil, sodium nitroprusside, uterine artery

From the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Iowa State University.
Supported in part by the American Heart Association (Iowa Affiliate) and National Institutes of Health Grant No. HL 42567. Dr. Isla was supported by a scholarship (FPI, Ministerio de Educacion y Ciencia, Spain).
Received for publication August 29, 1989; revised May 18, 1990; accepted June 15, 1990.
Reprint requests: D. C. Dyer, PhD, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Iowa State University, Ames, IA 50011.
6/1/23236

Nifedipine, methoxyverapamil (D-600, A. G. Knoll Pharmaceutical Laboratories, Ludwigshafen, West Germany), and sodium nitroprusside have been used in human beings to counteract pregnancy-induced hypertension.[1, 2, 3] The mechanisms responsible for the vasodilation effects of these agents are not clearly established. Nifedipine is a dihydropiridine Ca^{++} antagonist that has marked selectivity for potential dependent Ca^{++} channels in vascular smooth muscle.[4] D-600 has approximately equiactive effects in blocking poten-

tial dependent Ca^{++} channels in cardiac muscle and vascular smooth muscle,[5] and has been recently shown to interact with certain neurotransmitter receptors, specifically serotonin receptors.[6-8] On the other hand, nitrovasodilators such as sodium nitroprusside interfere with contractile responses due to receptor stimulation, which putatively increases myoplasmic free Ca^{++} either by releasing intracellularly stored Ca^{++} or by acting directly on Ca^{++} channels linked to the receptor.[9]

The pregnant ewe is widely used as an experimental animal in the study of maternal-fetal physiology-pharmacology. We recently showed in the late pregnant ewe isolated uterine artery that norepinephrine and serotonin induce long-lasting, strong vasoconstrictor responses through the stimulation of α_1-adrenergic and $5HT_2$ (serotonergic) receptors, respectively.[10, 11] A pronounced vasoconstriction in the uterine circulation induced by these agonists could have a detrimental effect on the proper functioning of reproductive organs and perfusion of the fetus as in pregnancy-induced hypertension.

In this study we compare the effects of nifedipine, D-600, and sodium nitroprusside on contractions induced by norepinephrine, serotonin, and K^+ depolarization with the aim of providing additional information for the clarification of the vasoinhibitory action of these agents. Particular attention is given to the determination of the Ca^{++} source for the agonist producing the smooth muscle contraction, inasmuch as the final determinant of vasoconstriction appears to be an increase in intracellular free Ca^{++}.

Material and methods

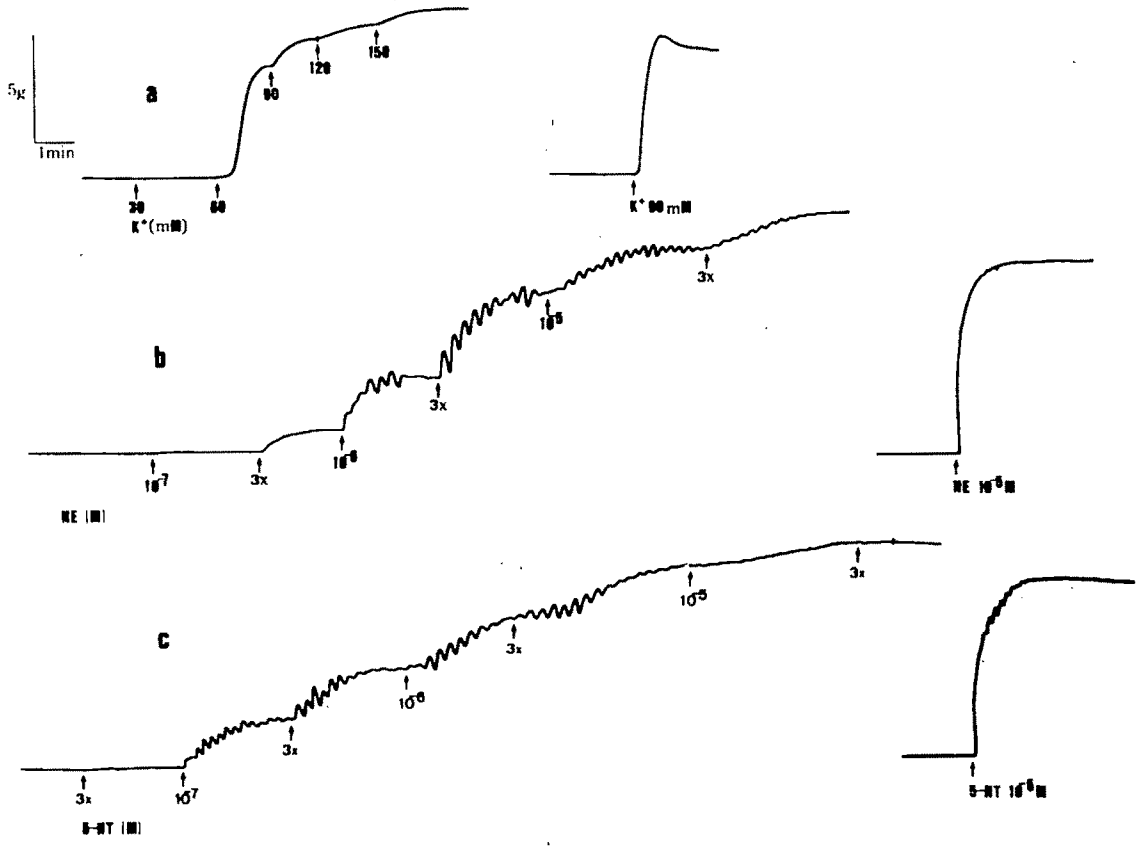
Specimens of uterine arteries were obtained from pregnant ewes within 2 weeks of term (131 to 145 days' gestation). Animals were killed by intravenous injection of pentobarbital and exsanguination. Immediately after exsanguination, sections of the mesometrium and uterus that contain the uterine arteries were placed in ice-cold oxygenated modified Krebs-Henseleit solution and taken to the laboratory. The Krebs-Henseleit solution had the following composition (mmol/L): sodium chloride 115.21, sodium bicarbonate 22.14, potassium chloride 4.7, magnesium sulfate 1.16, potassium dihydrogen phosphate 1.2, ethylenediaminetetraacetic acid ($2H_2O$) 0.003, dextrose 7.88, and calcium chloride 1.8. The uterine arteries were dissected from the adhering tissues and isolated arterial rings were prepared as described previously.[12] In brief, rings with an outer diameter of 3 to 4 mm and a width of about 3 mm were obtained from the main uterine artery before the first bifurcation. Preparations were mounted between two stainless-steel hooks in 10 ml organ baths containing Krebs-Henseleit solution at 37° C and oxygenated with a mixture of 5% carbon dioxide and 95% oxygen. Changes in isometric force were monitored by transducers (Grass FTO3C, Grass Instrument Co., Quincy, Mass.) and recorded on a polygraph (Grass model 79D) or a Beckman recorder.

The preparations were allowed to equilibrate during a period of 90 minutes. During this time the tissues were washed every 10 to 15 minutes and basal tension was repeatedly adjusted until the resting tension became stable at 1 *g*. A contraction to K^+ 90 mmol/L was obtained after the 90 minutes of equilibration and served as a control for the reproducibility of the contractions and as an internal standard for each vessel segment.[13]

Cumulative dose-response curves to norepinephrine (10^{-8} to 3×10^{-5} mol/L), serotonin (10^{-8} to 3×10^{-5} mol/L), and K^+ (9 to 150 mmol/L) were obtained. Single concentrations of the vasoconstrictors (norepinephrine, 10^{-5} mol/L, serotonin, 10^{-5} mol/L, K^+ 90 mmol/L) were used in studies with vasoinhibitors because the responses to these vasoconstrictors were reproducible during the course of the experiment.

The effect of calcium removal on the contractions induced by norepinephrine (10^{-5} mol/L), serotonin (10^{-5} mol/L), and K^+ (90 mmol/L) was ascertained by omitting calcium chloride from the Krebs-Henseleit solution. Tissues were incubated for 30 minutes in the Ca^{++}-free Krebs solution containing 0.1 mmol/L ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) (rinsed every 10 minutes) before the application of a vasoconstrictor. This procedure was repeated until the residual response to the vasoconstrictor was completely abolished. Then in the presence of a vasoconstrictor, Ca^{++} was added to the bath in a dose-dependent fashion (0.25 to 2.5 mmol/L) to restore contractile activity of the tissue.

Two different procedures were used to examine the effects of nifedipine, D-600, and sodium nitroprusside on the contractile responses to norepinephrine, serotonin, and K^+. In one group of experiments, after contractile control-responses to the vasoconstrictors were obtained, the tissues were treated with the vasoinhibitors for 30 minutes and then stimulated with the same agent. The tissues were washed extensively for 45 minutes and the next higher concentration of the same vasoinhibitor was added. This procedure was repeated until the vasoinhibitor dose-response curves were completed. In the other procedure, when the contractile responses to norepinephrine or serotonin had stabilized, a vasoinhibitor was added to the bath in increasing concentrations to ascertain its effect on the precontracted arteries. To reduce sites of loss (neuronal uptake and metabolism by catechol-O-methyltransferase) of the amine agonists from the receptor sites in the arteries, cocaine (10^{-5} mol/L) and tropolone (10^{-5} mol/L) were added to the bath 20 min-


5g
1min
a
b
c
3x
3x
3x

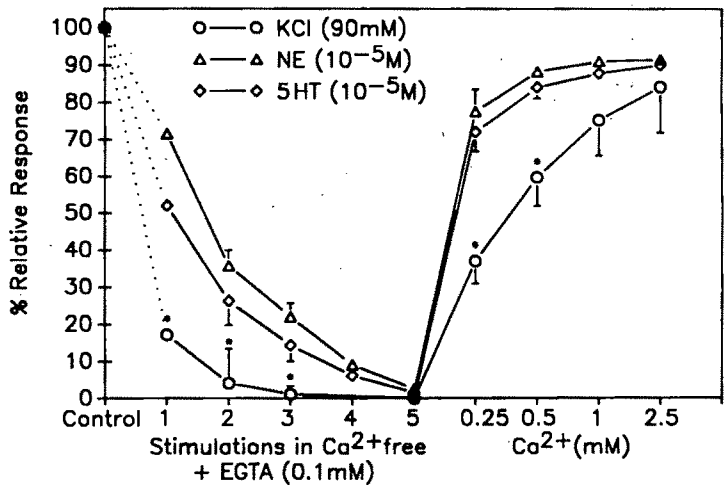


Fig. 2. Effect of replacement of Krebs-Henseleit solution (1.8 mmol/L Ca^{++}) with calcium-free solution containing EGTA (0.1 mmol/L) on the contractions induced by norepinephrine *(NE)* 10^{-5} mol/L, serotonin *(5-HT)* 10^{-5} mol/L, and K^+ 90 mmol/L. Tissues were stimulated every 30 minutes in calcium-free solution until responses were completely abolished. In presence of stimulating agents, calcium was then added to bath in dose-dependent fashion (0.25 to 2.5 mmol/L). Each point is mean ± SE of seven experiments. *Asterisks* indicate significant difference between corresponding points ($p < 0.05$).

norepinephrine required to produce 50% of the maximal response (ED_{50}) was 1.8 ± 0.3 × 10^{-6} mol/L. The addition of a single, submaximal concentration of norepinephrine (10^{-5} mol/L) to the tissue bath induced a fast and stable contraction of the uterine artery (160.0% ± 6.2% of K^+ 90 mmol/L; $n = 10$). The contraction to norepinephrine (10^{-5} mol/L) remained stable for more than 15 minutes (Fig. 1, *b*).

Serotonin (10^{-7} – 10^{-4} mol/L) induced concentration-dependent contractions. The concentration of serotonin required to produce 50% of its maximal response (11.3 ± 0.7 mN; $n = 10$) was 4.4 ± 0.4 × 10^{-7} mol/L and the maximal response to that of the K^+ maximum was 156.3% ± 9.1%, $n = 10$. A submaximal concentration of serotonin (10^{-5} mol/L) induced a contraction that was fast in onset and maintained a stable level of tension (143.3% ± 5.1% of K^+ 90 mmol/L, $n = 10$) for >15 minutes (Fig. 1, *c*).

Calcium sources. Calcium removal from the Krebs-Henseleit solution and the addition of EGTA (0.1 mmol/L) progressively reduced the response to the vasoconstrictors. After the second stimulation with K^+ (90 mmol/L) or the fourth stimulation with norepinephrine (10^{-5} mol/L) or serotonin (10^{-5} mol/L), the contractile response to each of the respective vasoconstrictors was completely abolished (Fig. 2). The subsequent administration of Ca^{++} back to the Krebs-Henseleit solution caused the tissue to regain activity to the vasoconstrictors, even to small concentrations of Ca^{++} (0.25 mmol/L). This recovery was more marked ($p < 0.05$) when norepinephrine or serotonin were the agonists than when the tissues were depolarized with potassium (Fig. 2).

Effect of pretreatment with nifedipine, D-600, and sodium nitroprusside. The effect of pretreatment of the tissues with the vasodilators against the contractile responses of norepinephrine (10^{-5} mol/L), serotonin (10^{-5} mol/L), and K^+ (90 mmol/L) is represented in Figs. 3, *A*, 4, *A*, and 5, respectively. The collected ID_{50} values and relative potencies are presented in Table I.

Nifedipine was more active ($p < 0.05$) in inhibiting K^+ than norepinephrine, and serotonin induced vasoconstriction. D-600 more effectively inhibited ($p < 0.05$) K^+- and serotonin-induced contractions than norepinephrine-induced vasoconstrictions, whereas sodium nitroprusside was equieffective against the three agonists.

Effect of nifedipine, D-600, and sodium nitroprusside on precontracted arteries. The vasodilators in a concentration-dependent manner relaxed arteries precontracted with either norepinephrine (10^{-5} mol/L) or serotonin (10^{-5} mol/L). The potency order for inhibiting either norepinephrine or serotonin precontracted rings was the same: sodium nitroprusside, D-600, nifedipine (Figs. 3, *B*, and 4, *B*). The ID_{50} values, the relative potency of the vasodilators, and the maximum inhibitory effect are presented in Table II.

A comparison of the inhibitory effects of the vasodilators on pretreated versus precontracted arteries indicated that D-600 was more active ($p < 0.05$) in inhibiting norepinephrine and serotonin vasoconstrictions when preincubated with the tissues than when

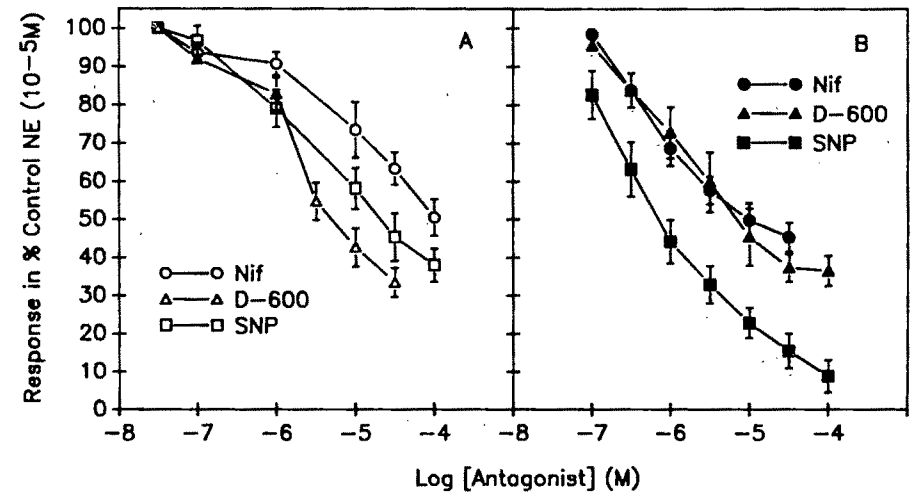


Fig. 3. Inhibitory effect of nifedipine *(Nif)*, methoxyverapamil *(D-600)*, and sodium nitroprusside *(SNP)* on isolated ring segments of late pregnant ovine uterine arteries contracted by 10^{-5} mol/L norepinephrine. **A**, Vasodilators were incubated for 30 minutes before re-adding norepinephrine *(open symbols)*. **B**, Vasodilators were administered in increasing concentrations to norepinephrine-precontracted arteries *(filled symbols)*. *Vertical bars* represent SE of five to seven experiments.

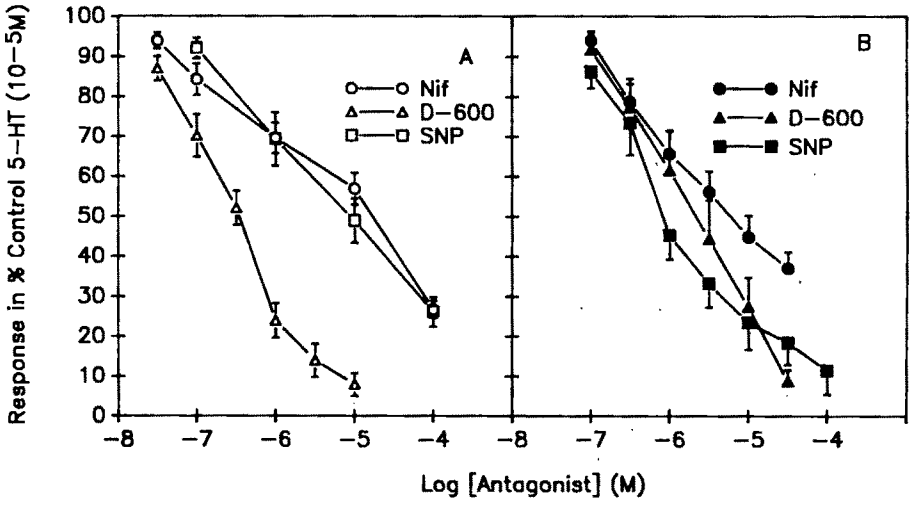


Fig. 4. Inhibitory effect of nifedipine *(Nif)*, methoxyverapamil *(D-600)*, and sodium nitroprusside *(SNP)* on isolated ring segments of late pregnant ovine uterine arteries contracted by 10^{-5} mol/L serotonin. **A**, Vasodilators were incubated 30 minutes before re-adding serotonin *(open symbols)*. **B**, Vasodilators were administered in increasing concentrations to serotonin-precontracted arteries *(filled symbols)*. *Vertical bars* represent SE of five to seven experiments.

administered to the precontracted arteries, whereas sodium nitroprusside when administered to the norepinephrine- and serotonin-precontracted arteries was significantly more effective ($p < 0.05$) (Figs. 3 and 4).

Comment

The results in the present study illustrate the differential inhibitory effect of nifedipine, D-600, and sodium nitroprusside on contractile responses to norepinephrine, serotonin, and potassium. Potassium-induced (90 mmol/L) contractions were completely abolished after the second stimulation in the Ca^{++}-free medium. The contraction gradually recovered when Ca^{++} was readded to the solution, indicating a complete dependency on extracellular Ca^{++} of the contraction to K^+. Nifedipine was very active in antagonizing the K^+-depolarized response of uterine blood vessels. Because nifedipine has a marked selectivity for potential dependent Ca^+ channels in vascular smooth muscle,[4] it could be suggested that in the uterine artery Ca^{++} influx through these channels is essential for the contraction of K^+ depolarization. The lack of effect of

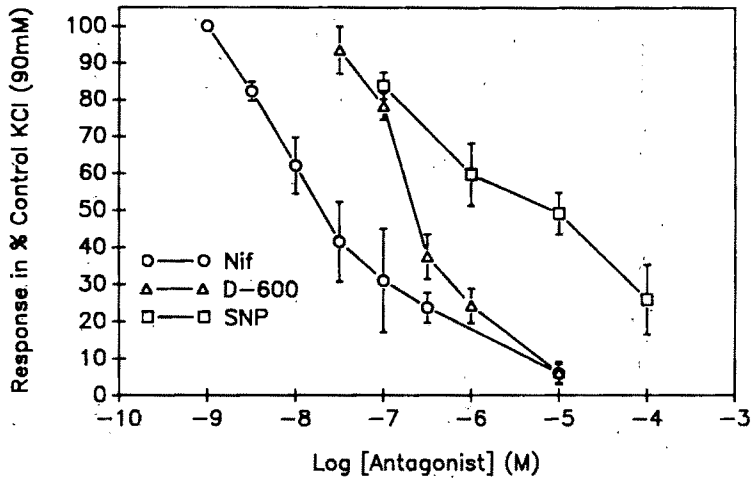


Fig. 5. Inhibitory effect of nifedipine *(Nif)*, methoxyverapamil *(D-600)*, and sodium nitroprusside *(SNP)* on isolated ring segments of late pregnant ovine uterine arteries contracted by 90 mmol/L potassium chloride. Tissues were equilibrated with the vasodilators for 30 minutes before addition of potassium chloride. *Vertical bars* represent SE of five experiments.

phentolamine (10^{-7} mol/L) on the K^+-induced contraction suggests that K^+ does not release norepinephrine from adrenergic nerve terminals in the ovine uterine artery at term as was found by Fallgren and Edvinsson[13] in the guinea pig uterine artery, and might, in part, explain the decay of the tonic contraction to K^+ (90 mmol/L) depolarization.

On the other hand, norepinephrine- and serotonin-induced contractions were much more resistant to Ca^{++} removal. The addition of minimal quantities of Ca^{++} (0.25 mmol/L) produced an almost complete recovery of the maximal contractile effect to norepinephrine and serotonin. This suggests a high calcium sensitivity of the contractile system in the arterial smooth muscle of the ovine uterine artery from pregnancy. As has been shown in the human[16] and guinea pig uterine artery[17] vasoconstriction relies mainly on the presence of extracellular calcium and in the case of drug-induced contractile responses also on an intracellular pool of calcium.

Nifedipine was the least active of the three vasodilators in inhibiting the contractions induced by norepinephrine or serotonin. Neither pretreatment of the tissues with the highest concentrations of nifedipine (10^{-4} mol/L) nor administration of nifedipine (10^{-8} to 10^{-4} mol/L) to the precontracted arteries completely abolished norepinephrine- and serotonin-induced vasoconstriction. These results are consistent with the findings that norepinephrine and serotonin activate respectively α_1-adrenergic and $5HT_2$ (serotonergic) receptors[10, 11] and presumably Ca^{++} influx occurs through receptor operated nifedipine insensitive Ca^{++} channels. It might be suggested that the use of nifedipine for the treatment of pregnancy-induced hypertension requires further study because even though it might decrease maternal blood pressure it does not block the vasoconstrictor response to norepinephrine and serotonin and therefore would not be expected to cause vasodilatation in the uterine vasculature if these amines were involved in the hypertension. This observation is in agreement with the results obtained by Veille and coworkers[18] with pregnant animal models in which nifedipine did not alter the uterine flow appreciably and did not cause excessive hypotension. However, as a second-line antihypertensive drug in pregnancy in a slow release preparation, nifedipine has been found to be an effective antihypertensive in human patients.[3]

In this study when D-600 was incubated with the uterine arteries, the contractile responses to serotonin and K^+ were more effectively inhibited than those to norepinephrine. D-600, when preequilibrated with the tissue, was 30 times more active than nifedipine and sodium nitroprusside in inhibiting the serotonin-induced vasoconstriction, whereas it was 10 times less active than nifedipine in inhibiting the contraction to K^+ depolarization. These results taken together suggest that D-600 is very active in blocking serotonin-induced vasoconstrictions. These results are in agreement with those of Auguet and colleagues[7, 8] in studies on isolated rabbit aorta in which they found that D-600 and verapamil in addition to blocking potential dependent calcium channels can also interact with certain neurotransmitter receptors and are very potent competitive antagonists of serotonin-induced vasoconstrictions. In the uterine artery, D-600 seems to be somewhat more selective in blocking serotonin receptors and therefore it might be best characterized as a Ca^{++}-serotonin an-

Table I. Inhibitory effect of nifedipine, sodium nitroprusside, and D-600 on the contractile responses to norepinephrine (10^{-5} mol/L), serotonin (10^{-5} mol/L), and high extracellular potassium (90 mol/L) in the late pregnant ovine uterine artery

	Norepinephrine (10^{-5} mol/L)		*Serotonin (10^{-5} mol/L)*		*K^+ (90 mmol/L)*	
	ID_{50} (mol/L)	*Relative potency*	*ID_{50} (mol/L)*	*Relative potency*	*ID_{50} (mol/L)*	*Relative potency*
Nifedipine	$1.38 \pm 0.4 \times 10^{-4}$	0.11	$2.04 \pm 0.4 \times 10^{-5}$	0.036	$1.95 \pm 0.9 \times 10^{-8}$*	11.64
Sodium nitroprusside	$2.05 \pm 0.5 \times 10^{-5}$	0.59	$7.80 \pm 0.5 \times 10^{-6}$	0.031	$8.40 \pm 0.7 \times 10^{-6}$	0.09
D-600	$4.98 \pm 0.6 \times 10^{-6}$*	1.00	$3.30 \pm 0.3 \times 10^{-7}$	1.000	$2.15 \pm 0.4 \times 10^{-7}$	1.00

The vasodilators were allowed to equilibrate with the tissues 30 minutes before a subsequent stimulation was obtained. The concentration of vasodilator reducing the maximum contractile effect to 50% (ID_{50}) was determined graphically by linear interpolation. Relative potency of the vasodilators was determined by the method of the 2-and-2 dose assay[15] by assigning D-600 a potency of 1. Values are mean ± SE of five to seven different animals.

*Significant differences of a vasodilator between vasoconstrictors ($p < 0.05$).

Table II. Inhibitory effect of nifedipine, sodium nitroprusside, and D-600 on the contractile responses to norepinephrine and (10^{-5} mol/L) and serotonin (10^{-5} mol/L) in the late pregnant ovine uterine artery

	Norepinephrine			*Serotonin*		
	ID_{50} (mol/L)	*Imax (%)*	*Relative potency*	*ID_{50} (mol/L)*	*Imax (%)*	*Relative potency*
Nifedipine	$9.6 \pm 0.3 \times 10^{-6}$	54.8 ± 3.9	0.98	$5.8 \pm 0.4 \times 10^{-6}$*	37.0 ± 4.1	0.44
Sodium nitroprusside	$7.1 \pm 0.4 \times 10^{-7}$	93.1 ± 2.5	11.02	$8.2 \pm 0.6 \times 10^{-7}$	83.8 ± 5.4	1.47
D-600	$6.9 \pm 0.5 \times 10^{-6}$	63.6 ± 4.0	1.00	$4.7 \pm 0.9 \times 10^{-6}$*	91.3 ± 2.9	1.00

The vasodilators were administered at increasing concentrations to the precontracted arteries. The concentration of vasodilator relaxing the precontracted artery to the 50% (ID_{50}) level was determined graphically by linear interpolation. *Imax* refers to the greatest inhibition observed to that vasodilator. Relative potency of the vasodilators was determined by the method of the 2-and-2 dose assay,[15] by assigning D-600 a potency of 1.

*Significant difference between the same treatment for the two vasoconstrictors ($p < 0.05$).

tagonist rather than as a pure Ca^{++} entry blocker. Accordingly, the significantly higher inhibitory effect of D-600 on the phasic rather than on the sustained component of the serotonin-induced contraction could be explained by its competitive antagonism on the serotonin receptor. The combined Ca^{++}-serotonin antagonism might be of interest in the treatment of pregnancy-induced hypertension. In this sense Serafini and collaborators[1] reported that D-600 administered intravenously to patients decreased the maternal blood pressure without compromising the fetus.

On the other hand, the sustained rather than the phasic component of the serotonin- or norepinephrine-induced contraction was more effectively blocked ($p < 0.05$) by sodium nitroprusside. This differential inhibitory effect of sodium nitroprusside might indicate that several Ca^{++} pathways can be activated by these agonists during the course of the contraction. In this sense it has been suggested in vascular smooth muscle that contractile responses to vasoactive agonists such as norepinephrine and serotonin are initiated by intracellular Ca^{++} release and maintained by an influx of extracellular Ca^{++}.[19, 20] Hence, in the uterine artery, sodium nitroprusside, seems to be blocking the influx of extracellular Ca^{++} rather than affecting the intracellular Ca^{++} pools. It is of particular interest to note that sodium nitroprusside can effectively inhibit the sustained contractions to norepinephrine and serotonin in the isolated ovine uterine artery, whereas a sustained contraction of the uterine vasculature in vivo might compromise the fetus.

Contradictory results have been obtained when sodium nitroprusside was administered to reverse hypertension in chronically instrumented pregnant ewes. Some animals required high rates of infusion of sodium nitroprusside (25 μg/kg/min)[21, 22]; whereas for others, low rates of infusion (<1 to 3 μg/kg/min) were enough to maintain the desired 20% decrease in mean maternal pressure.[22, 23] In the former no change or decreased uterine blood flow was reported and fetal death occurred within 30 minutes of cessation of sodium nitroprusside infusion because of cyanide toxicity. However, in the latter the safety of this therapy for both mother and fetus was shown. Whereas caution must be taken in the extrapolation of data from in vitro studies, our results with sodium nitroprusside in isolated late pregnant ovine uterine artery confirm the results obtained by Nelson and Suresh[24] in pregnant and non-

pregnant human uterine arteries and provide a rationale for the use of sodium nitroprusside in hypertensive emergencies in pregnant patients if standard therapy has failed.

In conclusion, the results of this study show that nifedipine, D-600, and sodium nitroprusside induce dilatation of uterine arteries from near-term pregnany ewes: (1) nifedipine effectively inhibits K^+-induced contractions but is relatively ineffective in blocking norepinephrine- and serotonin-induced contractions; (2) D-600 is more effective on the phasic than on the tonic component of the contractions to norepinephrine and serotonin and is specifically active in inhibiting serotonin-induced contractions, being best characterized in this tissue as a Ca^{++}-serotonin antagonist rather than as a pure Ca^{++} entry blocker; and (3) sodium nitroprusside preferentially inhibits the sustained tonic component of norepinephrine- and serotonin-induced vasoconstrictions.

REFERENCES

1. Serafini PC, Petracco A, Vicosa HM, Costa PL. Efeito hipotensor arterial do verapamil na pre-eclampsia grave. Estudo preliminar. Arq Braz Cardiol 1979;32:57-64.
2. Stemple JE, O'Grady JP, Morton MJ, Johnson KA. Use of sodium nitroprusside in complications of gestational hypertension. Obstet Gynecol 1982;60:533-8.
3. Constantine G, Beevers DG, Reynolds AL, Luesley DM. Nifedipine as a second line antihypertensive drug in pregnancy. Br J Obstet Gynaecol 1987;94:1136-42.
4. Spedding M. Calcium antagonist subgroups. Trends Pharmacol Sci 1985;6:109-13.
5. Fleckenstein A. Fundamental actions of calcium antagonists on myocardial and cardiac pacemaker cell membranes. In: Weiss GB, ed. New perspectives of calcium antagonists. Baltimore: Williams and Wilkins, 1981:59.
6. Karliner JS, Motulsky HJ, Dunlap J, et al. Verapamil competitively inhibits α_1-adrenergic and muscarinic but not β-adrenergic receptors in rat myocardium. J Cardiovasc Pharmacol 1982;4:515-20.
7. Auguet M, DeFedulis FV, Clostre F. Competitive inhibition of 5-HT receptors in rabbit isolated aorta by the CA^{2+}-antagonist methoxyverapamil (D-600). Neurochem Int 1984;6:701-10.
8. Auguet M, DeLaFlotte S, Clostre F, DeFedulis FV. Verapamil as an apparent competitive antagonist of the serotonin receptor of rabbit isolated aorta. Gen Pharmacol 1986;17:133-5.
9. Shibata S, Satake N, Hester RK. Differential inhibitory effects of nitroglycerin on contractile responses to the α-adrenoceptor agonists, methoxamine and clonidine, in rabbit aorta. J Cardiovasc Pharmacol 1989;13:245-52.
10. Isla M, Dyer DC. Characterization of α-adrenergic receptors in the late pregnant ovine uterine artery. Eur J Pharmacol 1990;178:321-31.
11. Zhang L, Dyer DC. Characterization of 5-hydroxytryptamine (5-HT) receptors on isolated ovine uterine artery in late pregnancy. J Pharmacol Exp Ther 1990;253:1236-44.
12. Suresh MS, Nelson SH, Nelson TE, Steinsland OS. Pregnancy: increased effect of verapamil in human uterine arteries. Eur J Pharmacol 1985;112:387-91.
13. Fallgren B, Edvinsson L. Characterization of adrenoceptor mechanisms in isolated guinea-pig uterine arteries. Eur J Pharmacol 1986;131:163-70.
14. Innes IR. An action of 5-hydroxytryptamine on adrenaline receptors. Br J Pharmacol 1962;19:427-33.
15. Tallarida RJ, Murray RB, eds. Manual of pharmacologic calculations with computer programs. 2nd ed. New York: Springer Verlag, 1987.
16. Kostrzewska A, Landanski T, Batra S. Effect of calcium and calmodulin antagonists on contractile responses of the human uterine artery. Br J Pharmacol 1988;94:1037-42.
17. Fallgren BO, Bergstrand H, Edvinsson L. Calcium influx and protein kinase C activation involved in uterine vasoconstriction in guinea pigs. Eur J Pharmacol 1989; 170:61-7.
18. Veille JC, Bissonnette JM, Hohimer AR. The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat. Am J Obstet Gynecol 1986;154:1160-3.
19. Khalil RA, Van Breemen C. Sustained contraction of vascular smooth muscle: calcium influx or C-kinase activation? J Pharmacol Exp Ther 1988;244:537-42.
20. Berridge MJ. Inositol triphosphate and diaclyglycerol as second messengers. Biochem J 1984;220:345-60.
21. Lieb SM, Zugaib M, Nuwayhid B, et al. Nitroprusside-induced hemodynamic alterations in normotensive and hypertensive pregnant sheep. Am J Obstet Gynecol 1981;139:925-31.
22. Naulty J, Cefalo RC, Lewis PC, Fetal toxicity of nitroprusside in the pregnant ewe. Am J Obstet Gynecol 1981;138:708.
23. Ellis S, Wheeler MD, James FM, et al. Fetal and maternal effects of sodium nitroprusside used to counteract hypertension in gravid ewes. Am J Obstet Gynecol 1982;143:766.
24. Nelson SH, Suresh MS. Comparison of nitroprusside and hydralazine in isolated uterine arteries from pregnant and nonpregnant patients. Anesthesiology 1988;68:541-7.

Fetal and maternal response to intravenous infusion of a thromboxane synthetase inhibitor

John P. Cardin, Jr., MD,[a] Michael G. Ross, MPH, MD,[a] M. Gore Ervin, PhD,[a] A. Victoria Schaffer, MD,[b] Frank L. Douglas, PhD, MD,[b] and John P. Simke[b]

Torrance, California, and Summit, New Jersey

Pharmacologic inhibition of thromboxane synthetase activity has reversed the clinical manifestations of toxemia in the ovine model. To investigate placental transfer and fetal effects of a selective thromboxane synthetase inhibitor, CGS13080 (Ciba-Geigy, Summit, N.J.) was intravenously infused into eight singleton- or twin-bearing ewes near term. During CGS 13080 infustion (0.1 mg/kg/hr), maternal steady-state CGS 13080 levels of 102 ± 18 ng/ml were achieved within 30 minutes and maternal serum thromboxane generation decreased significantly (13 ± 3 to 4 ± 1 ng/ml). However, fetal serum levels of CGS 13080 were only 4% of peak maternal concentrations and fetal serum thromboxane generation did not change. There was no evidence of change in uterine blood flow, maternal or fetal blood pressure, heart rate, blood gas values, or fetal or maternal metabolites of prostacyclin or prostaglandin E_2 during the study. We speculate that CGS 13080 may be efficacious in the treatment of human pregnancy-induced hypertension. (AM J OBSTET GYNECOL 1990;163:1345-9.)

Key words: Pregnancy-induced hypertension, thromboxane synthetase inhibitor, pregnancy, CGS 13080, sheep

Pregnancy-induced hypertension is a leading cause of maternal and perinatal morbidity and mortality and occurs in approximately 6% of all pregnancies.[1] Although the precise cause of pregnancy-induced hypertension is unknown, alterations in prostaglandin metabolite production are believed to be central to the pathophysiology. Several investigators have suggested that a prostacyclin deficiency occurs in both maternal and fetal circulations.[2-7] This may result in an imbalance in the prostacyclin-to-thromboxane ratio and account for the many clinical manifestations of pregnancy-induced hypertension. Recent evidence indicates that selective thromboxane synthetase inhibitors may be efficacious in the treatment of pregnancy-induced hypertension. In several case reports women with pregnancy-induced hypertension showed clinical improvement in response to infusion of a thromboxane synthetase inhibitor.[8, 9] In an ovine toxemia model (starvation-induced) Keith et al.[10] demonstrated complete reversal of the pathophysiologic changes and a significant increase in plasma prostacyclin levels after intravenous thromboxane synthetase inhibitor administration. Although a thromboxane synthetase inhibitor may have clinical efficacy, there is limited knowledge of the effects of maternal infusion on the fetus.

The purpose of this study was to quantitate the placental transfer of the thromboxane synthetase inhibitor CGS 13080 (Ciba-Geigy, Summit, N.J.) when infused to the maternal ewe and to determine the effects of CGS 13080 on both ovine maternal and fetal prostaglandin levels during maternal drug infusions.

Material and methods

Eight Western mixed-breed ewes with both singlet (n = 5) and twin (n = 3) fetuses were studied at a mean (± SEM) gestation of 132 ± 2 days. Guidelines for the care and use of animals approved by Harbor–University of California at Los Angeles Medical Center were followed. The animals were housed indoors in individual steel cages and were acclimated to surroundings for at least 4 days before operation. All animals were operated on between 124 and 130 days' gestation and allowed 4 to 5 days of recovery before study.

At operation, anesthesia was induced by an intramuscular injection of ketamine hydrochloride (750 mg) followed by continuous intravenous infusion of ketamine (600 mg/hr) and locally applied lidocaine (1%). After sterile preparation and draping, a midline incision exposed both uterine horns. After the position of

From the Departments of Obstetrics and Gynecology and Pediatrics, Harbor–University of California at Los Angeles, Torrance,[a] and the Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit.[b]

This work was supported in part by Ciba-Geigy Corporation, Summit, New Jersey.

Received for publication December 12, 1989; accepted June 15, 1990.

Reprint requests: Michael Ross, MPH, MD, Department of Obstetrics and Gynecology, RB No. 1, Harbor-UCLA Medical Center, 1124 West Carson St., Torrance, CA 90509.

6/1/23226

the fetus was determined, a single hind leg was exposed through a small hysterotomy. Polyethylene catheters (inside diameter, 1.0 mm; outside diameter, 1.8 mm) were inserted in the dorsal hind limb vein and artery and threaded to the inferior vena cava and abdominal aorta, respectively. Before hysterotomy closure, a plastic catheter (Corometrics Medical System, Inc., Wallingford, Conn.) was sutured to the fetal hind limb. If twins were present, the above procedure was repeated for the second fetus. In four animals the left uterine artery was isolated and an electromagnetic flow probe (Dienco, Los Angeles) was sutured in place. All catheters were exteriorized to the maternal flank and placed in a cloth pouch sewn to the ewe's flank. The maternal artery and vein were catheterized at the femoral triangle and threaded to the descending aorta and inferior vena cava, respectively. To maintain catheter patency, the fetal vascular catheters were filled with 1000 U/ml of sodium heparin and maternal catheters with 10 U/ml of sodium heparin. Catheters were sealed with sterile caps and flushed daily. Ewes were administered intravenous gentamicin 80 mg, chloromycetin 600 mg, and oxacillin 500 mg before operation and twice daily for 3 days after operation.

Experimental protocol. Experiments were performed on unanesthetized animals after a minimum of 5 days of recovery. Animals were studied in the same individual cages in which they were maintained. All animals were weighed before drug infusion. The thromboxane synthetase inhibitor imidazo-(1,5-2)-pyridine-5-hexanoic acid (CGS 13080) obtained in a powdered form was dissolved in water to achieve a final concentration of 0.67 mg/ml. To effectively dissolve CGS 13080 into solution, the pH was adjusted to 7.4 with 50 mmol/L Tris base and the temperature raised to 40° to 45° C.

The study design included a 30-minute control period (−30 to 0 minutes), a 3-hour infusion (0 to 180 minutes) of CGS 13080 (0.1 mg/kg/hr) into the maternal venous catheter and a recovery phase (180 to 360 minutes). Fetal and maternal arterial blood samples (2.5 ml) were obtained at −30, 0, 30, 60, 120, 180, 240, 300, and 360 minutes. In two of the three pairs of twins both fetuses were sampled and monitored. An arterial blood sample (1 ml) was collected at each time interval and allowed to clot at 37° C for 1 hour before centrifugation. Serum was stored at −20° C until assayed for the serum prostaglandin metabolites prostaglandin E_2 (PGE_2), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), and thromboxane B_2 (TXB_2). In addition, 1 ml of blood was collected in 10 μl of 0.1mol/L lithium heparin, and an aliquot was taken to measure the hematocrit before centrifugation. The plasma was stored at −20° C until assayed for CGS 13080. Finally, 0.5 ml of blood was collected in a heparinized tuberculin syringe for immediate measurement of pH, Po_2, and Pco_2. Blood gases and pH were determined at 39° C with a Bm 33 MK2-PHM 72 MKS acid-base analyzer system (Radiometer Co., Copenhagen). After each sample, the fetal blood was replaced with an identical volume of maternal heparinized blood withdrawn before the study. Maternal samples were replaced with saline solution. Fetal and maternal arterial blood pressure, heart rate, and amniotic pressures were continuously monitored with an R-612 physiologic recorder (Beckman, Palo Alto). Fetal pressures were corrected for amniotic fluid pressures.

Determination of plasma CGS 13080 concentration. CGS 13080 concentrations were determined by high-performance liquid chromatography analysis.[11] Briefly, plasma samples (100 μl) were added with standard CGS 13080 (100 ng in 25 μl) and diluted with distilled water (1 ml). The mixture was loaded onto a 1 ml Bond Elut SCX minicolumn (Analytichem, Harbor City, Calif.) previously washed with distilled water (3 ml). CGS 13080 was then eluted with 2 ml of 4% ammonium hydroxide in methanol. The eluent was evaporated to dryness under nitrogen and reconstituted in 250 μl before high-performance liquid chromatography analysis. An aliquot (50 μl) of the extract was injected onto a Beckman Ultrasphere C18 analytical column. The flow rate was 1.5 ml/min and the eluent was monitored for fluorescence with a model 980 detector (The Anspec Co., Ann Arbor) equipped with a 418 nm filter. Calibration was performed with authentic CGS 13080 in blank human plasma samples. The limit of detection of CGS 13080 was 5 ng/ml. Standard curves were linear over the entire range of detection from 5 ng/ml to 3 μg/ml. The interassay variation was 3%. Chromatograms for controls (blank) plasma samples did not contain any peaks at the retention time for CGS 13080. Because of the limit of sensitivity, measurable background interference was noted in blank plasma samples. Background values obtained during the control period were subtracted from each value obtained during the infusion and recovery periods.

Eicosanoid assays. Measurements of serum TBX_2, 6-keto-$PGF_{1\alpha}$, and PGE_2 were performed by radioimmunoassay. Prostanoid metabolite standards were obtained from The Upjohn Company, Kalamazoo, and stock solutions of 1 mg/ml in 10% (v/v) acetonitrile in water were prepared, diluted to 100 ng/ml, and stored at 4° C. Dilutions of standard compounds ranging from 0.072 to 18 ng/ml or of unknown samples were made in 0.05 mol/L Tris hydrochloride (pH 6.8) containing 0.9% (sodium chloride, 0.3% bovine serum albumin, 0.05% sodium azide, and 0.01 mol/L ethylenediamine tetraacetic acid to construct a standard curve. The 50% binding level of the standard curve was 1.5 ng/ml. Iodine 125 methyl esters of TXB_2, 6-keto-$PGF_{1\alpha}$ and

PGE_2, all at 2200 Ci/mmol, were obtained from Advanced Magnetics, Inc., Cambridge, Mass. as were rabbit antisera to TXB_2, 6-keto-$PGF_{1\alpha}$, and PGE_2. Samples were assayed in duplicate. Standards or samples were mixed with ^{125}I-tracer (12,000 cpm), and the appropriate rabbit antiserum (all reagents at predetermined optimal levels) was added. The mixture was incubated for 16 hours at 4° C in 25 × 75 mm tubes, and then an excess of goat antirabbit antibody bound to paramagnetic particles was added. The magnetic complexes were separated by attraction to the tube wall by means of a magnetic rack. The bound radioactivity was determined with a Laboratory Technologies 25-well γ-counter. Sample dilutions were adjusted to give results falling between 20% and 80% of maximum binding; under these conditions the lowest quantifiable value was 0.3 ng/ml. Intraassay variation between replicates was 5% and total interassay variance was 12%.

Calculations and statistics. The steady-state maternal plasma CGS 13080 clearance rate was calculated as: Infusion rate of CGS 13080/(CGS 13080 concentration at steady state). Mean residence time was calculated as: Plasma volume/Plasma clearance rate, where the plasma volume was calculated on the basis of an assumed blood volume of 56 ml/kg for the adult ewe.[12]

All values are reported as mean ± SEM. Differences over time were assessed by one-way analysis of variance. If a significant F test was present, Dunnett's test was applied to compare control means to each group mean.

Results

In response to intravenous infusion of CGS 13080, the maternal plasma CGS 13080 levels increased to 102 ± 18 ng/nl. A steady-state concentration was achieved by 30 minutes of infusion. After discontinuation of the infusion, maternal plasma CGS 13080 decreased linearly to a nadir of 10 ± 6 ng/ml at 3 hours of recovery (Fig. 1). Fetal plasma CGS 13080 became detectable during maternal infusion (4.2 ± 0.5 ng/ml, $p < 0.05$) at levels approximately 4% of peak maternal values. However, fetal plasma CGS 13080 was nondetectable by the end of the 3-hour recovery period.

As expected, maternal serum TXB_2 decreased significantly (13.2 ± 2.9 to 3.6 ± 0.9 ng/ml, $p < 0.05$) during CGS 13080 infusion and the levels returned to near normal at the completion of the recovery phase. However, fetal serum TXB_2 did not change significantly ($p > 0.05$) during the study (Fig. 1). Maternal and fetal 6-keto-$PGF_{1\alpha}$ and PGE_2 levels did not change during the study (Fig. 1).

The steady-state plasma clearance was 16.3 ml/min/kg and the mean residence time was 2.80 minutes.

Maternal or fetal heart rate, mean arterial pressure, arterial blood gas, hematocrit, uterine blood flow, and amniotic fluid pressures did not change (Table I).

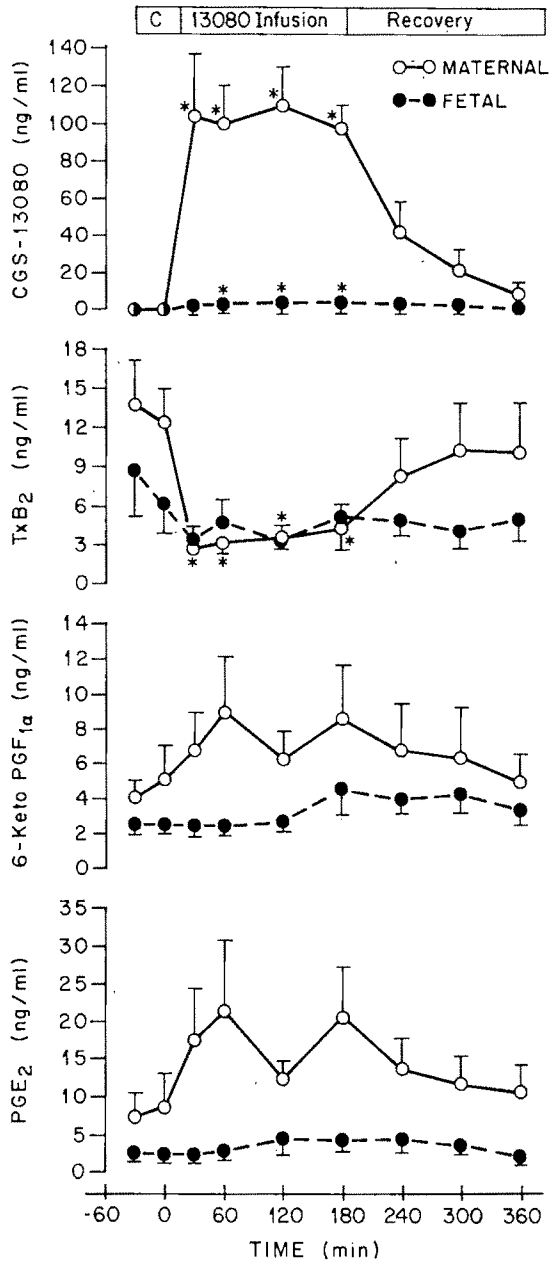


Fig. 1. Maternal and fetal plasma CGS 13080, TXB_2, 6-keto-$PGF_{1\alpha}$, and PGE_2 concentrations before, during, and after infusion of CGS 13080 to pregnant ewes. *Asterisk*, $p < 0.05$ versus mean basal value.

Comment

During normal pregnancy the production of prostacyclin increases by fivefold.[13] Available evidence supports the view that this increase contributes to the overall physiologic vasodilatation and decreased vascular resistance characteristics of early gestation. However, for unknown reasons a prostacyclin-deficiency state

Table I. Maternal and fetal arterial values (mean ± SEM) before, during, and after infusion of CGS 13080 to the maternal ewe

	Control	*Infusion*	*Recovery*
Maternal (n = 8)			
Hematocrit (%)	28.0 ± 0.5	28.0 ± 0.7	27.8 ± 0.4
pH	7.47 ± 0.01	7.47 ± 0.02	7.49 ± 0.01
Heart rate (beats/min)	118 ± 5	113 ± 3	114 ± 5
Mean arterial pressure (mm Hg)	78 ± 3	79 ± 2	83 ± 3
Uterine blood flow (ml/min)	304 ± 14	297 ± 10	312 ± 10
Fetal (n = 10)			
Hematocrit (%)	34.6 ± 1.1	34.7 ± 0.7	34.5 ± 0.8
pH	7.35 ± 0.02	7.35 ± 0.01	7.38 ± 0.02
Heart rate (beats/min)	166 ± 5	162 ± 3	161 ± 4
Mean arterial pressure (mm Hg)	53 ± 1	51 ± 1	52 ± 2
Amniotic fluid pressure (mm Hg)	5.9 ± 1.2	6.2 ± 1.2	7.0 ± 1.3

may develop in pregnancies predestined to have preeclampsia.[2-7] There is increasing evidence that patients with mild to severe pregnancy-induced hypertension show enhanced platelet activation and consumption[14] while placentas of patients with pregnancy-induced hypertension exhibit increased generation of thromboxane in vitro.[6] This results in an imbalance of thromboxane (vasoconstrictor) and prostacyclin (vasodilator). If the role of this imbalance is central to the pathophysiologic features of preeclampsia, then the use of selective inhibitors of thromboxane synthetase may be beneficial. Clinical studies with selective thromboxane synthetase inhibitors in pregnancy-induced hypertension are limited. An orally active thromboxane synthetase inhibitor (UK-37,248 or Dazoxiben, Pfizer Central Research, London) administered to 13 severely toxemic patients remote from term[8, 9] was shown to yield apparent clinical improvement. Six cases resulted in perinatal death attributed to prematurity and severe growth retardation, and seven resulted in live births.

The majority of research relative to the use of selective thromboxane synthetase inhibitors in pregnancy has used the ovine toxemia model as described by Thatcher and Keith.[15] Twin-bearing ewes starved for 72 hours develop many of the clinical manifestations of human toxemia. The clinical manifestations of hypertension, altered renal function and serum chemistry, and enhanced platelet aggregation can be reversed by use of selective thromboxane synthetase inhibitors (U-63,557A, The Upjohn Co., and CGS 13080).[10, 16]

CGS 13080 is a potent, selective inhibitor of thromboxane synthetase with an immediate onset of action and rapid clearance.[17] The in vitro potency of CGS 13080 as a thromboxane synthetase inhibitor is fivefold to sixfold greater than toward the other enzymes involved in arachidonic acid metabolism. Therefore the other prostaglandin endoperoxidase pathways are preserved. Although controversial, infusion of CGS 13080 to healthy humans does not result in a change from basal prostacyclin levels.[18-20] This is in accordance with our study, which demonstrated no significant change in maternal 6-keto-$PGF_{1\alpha}$ or PGE_2 during CGS 13080 infusion. However, an endoperoxide shunt may possibly occur in response to thromboxane synthetase inhibitors during pathologic conditions.[18]

Steady state was obtained within 30 minutes, with >75% inhibition of maternal serum thromboxane generation. Serum levels of thromboxane were reported because platelet aggregation ex vivo represents the most sensitive indicator of thromboxane generation.[21] As illustrated during the recovery phase, the administration of CGS 13080 resulted in a time- and dose-related inhibition of arachidonic acid–induced platelet aggregation ex vivo. The rapid clearance of CGS 13080 is consistent with data from nonpregnant human volunteers.[17] The drug is known to be metabolized to an inactive form in the liver, which is later excreted by the kidney.[17] Clinically, because of rapid plasma clearance, there is potential for titration during intravenous infusion.

This study demonstrated no effect on maternal or fetal blood pressure, heart rate, or uterine blood flow during the infusion. The absence of hypotension is in agreement with human studies that imply that thromboxane generation contributes little to the maintenance of systemic blood pressure during normotension. Unlike administration of a thromboxane synthetase inhibitor to the ovine toxemia model, which produced a rise in uterine blood flow,[10] our results suggest that uterine blood flow in the normotensive ewe may be independent of platelet thromboxane generation.

Significant CGS 13080 levels were found in fetal plasma during maternal infusions. However, these represented only 4% of peak maternal levels. CGS 13080 is known to be 96% protein-bound in plasma and poorly lipid-soluble,[17] factors that may account for the low fetal plasma levels. More important, these low fetal CGS 13080 levels were not associated with any significant changes in thromboxane generation from fetal platelets. These pharmacokinetics are limited to the ovine

model as there are significant placental structural differences in comparison with humans.[22]

We speculate that CGS 13080 may be potentially useful in treating and/or preventing pregnancy-induced hypertension. Preliminary reports suggest that prophylactic low-dose aspirin may be efficacious in the prevention of pregnancy-induced hypertension.[23, 24] Low-dose aspirin suppresses platelet thromboxane biosynthesis through inhibition of cyclooxygenase, which also could potentially affect the prostacyclin pathway.[25] The prostacyclin pathway is preserved during infusion of CGS 13080 under physiologic conditions.[18] Furthermore, the possibility exists that under pathologic conditions CGS 13080 may result in increased prostacyclin production as a result of an endoperoxide shunt. The armamentarium for the treatment of pregnancy-induced hypertension may include low-dose aspirin, thromboxane receptor inhibitors, or thromboxane synthetase inhibitors such as CGS 13080.

Special acknowledgement for their assistance in the preparation of this manuscript goes to Dr. Allan Wong and Ms. Barbara Barna-Andrusko for the plasma CGS 13080 measurements.

REFERENCES

1. Chesley LC. Hypertensive disorders in pregnancy. New York: Appleton-Century-Crofts, 1978 vol 20:1-15.
2. Remuzzi G, Marchesi D, Aoja C, et al. Reduced umbilical and placental vascular prostacyclin in severe preeclampsia. Prostaglandins 1980;20:105-10.
3. Downing L, Shepard GL, Lewis PJ. Reduced prostacyclin production in preeclampsia. Lancet 1980;2:1374.
4. Dadak C, Kefalides A, Sinzinger H, Weber G. Reduced umbilical artery prostacyclin formation in complicated pregnancies. AM J OBSTET GYNECOL 1985;144:792-5.
5. Walsh S, Behr M, Allen N. Placental prostacyclin production in normal and toxemic pregnancies. AM J OBSTET GYNECOL 1985;151:110-5.
6. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. AM J OBSTET GYNECOL 1985;152:335-40.
7. Fitzgerald D, Entman S, Mulloy K, Fitzgerald G. Decreased prostacyclin biosynthesis preceding the clinical manifestation of pregnancy-induced hypertension. Circulation 1987;75:956-63.
8. Van Assche FA, Spitz B, Vermylen J, Dechmijn H. Preliminary observations on treatment of pregnancy-induced hypertension with a thromboxane synthetase inhibitor. AM J OBSTET GYNECOL 1984;148:216-8.
9. Van Assche FA, Spitz B. Thromboxane synthetase inhibition in pregnancy-induced hypertension. AM J OBSTET GYNECOL 1988;159:1015-6.
10. Keith JC, Thatcher CD, Schaub RG. Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of pregnancy-induced hypertension. AM J OBSTET GYNECOL 1987;157:199-203.
11. Tripp SL, Rufino F. Assessment of the analytical method for CGS 13080 plasma analysis and concentrations of CGS 13080 in plasma from normal volunteers. Drug Metab Rep 83008, Ciba-Geigy Internal Report, 1983.
12. Coghlan JP, Fan JSK, Scoggins BA, Shulkes RA. Measurement of extracellular fluid volume and blood volume in sheep. Aust J Biol Sci 1977;30:71-84.
13. Goodman RP, Killam A, Brash AR, et al. Prostacyclin production during pregnancy: comparison of production during normal pregnancy and pregnancy complicated by hypertension. AM J OBSTET GYNECOL 1982;142:817-22.
14. Giles C, Inglis TCM. Thrombocytopenia and macrothrombocytosis in gestational hypertension. Br J Obstet Gynaecol 1981;88:1115-9.
15. Thatcher CD, Keith JC. Pregnancy-induced hypertension: development of a model in the pregnant sheep. AM J OBSTET GYNECOL 1986;155:201-7.
16. Miller KW, Keith JC. Erythrocyte morphologic features and serum chemistry studies in ovine pregnancy-induced hypertension treated with thromboxane synthetase inhibitors. AM J OBSTET GYNECOL 1988;159:1241-6.
17. Orientation brochure CGS 13080. Cardiovascular Drug Development. Summit, New Jersey: Ciba-Geigy.
18. Henriksson P, Rasmanis O, Edhag O, et al. Thromboxane synthase inhibition: "endoperoxide shunt phenomenon" does not occur in healthy humans in vivo. Prostaglandins 1990;39:99-107.
19. Fischer S, Struppler M, Bohlig B, et al. The influence of selective thromboxane synthetase inhibition with a novel imidazole derivative, UK-38,485, on prostanoid formation in man. Circulation 1983;68:821-6.
20. Green K, Vesterqvist O. In vivo synthesis of thromboxane and prostacyclin in man in health and disease. Data from GC-MS measurement of major urinary metabolites. Adv Prostaglandin Thromboxane Leukotriene Res 1986;16:309-24.
21. Fitzgerald GA, Brash AR, Oates JA, Pedersen AK. Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthetase in man. J Clin Invest 1983;72:1336-43.
22. Faber JJ, Kent TL. Permeability of the placental membrane for hydrophilic substances. In: Placental physiology. New York: Raven Press, 1983:79.
23. Beaufils M, Uzan S, Donsimoni R, Colau JC. Prevention of preeclampsia by early antiplatelet therapy. Lancet 1985;1:840-2.
24. Wallenburg HCS, Dekker GA, Makovitz JW, et al. Low-dose aspirin prevents pregnancy-induced hypertension and preeclampsia in angiotensin-sensitive primigravidae. Lancet 1986;1:1-3.
25. Fitzgerald GA, Oates JA. Selective and nonselective inhibition of thromboxane formation. Clin Pharmacol Ther 1984;35:633-40.

Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors

S. Devaskar, MD, K. McMenamy, MD, L. Holtzclaw, BS, and F. Sadiq, MD
St. Louis, Missouri

We investigated the effects of long-term (5 to 6 days) in vivo exposure to hyperinsulinemia and hypoinsulinemia on maternal and fetal rat (18 to 20 days' gestation; term ~ 21 days) brain and liver insulin receptors. Further we studied the in vitro effects of long-term insulin exposure on cultured rabbit neuronal cell insulin receptors. Long-term glucose infusions to maternal rats resulted in maternal and fetal hyperglycemia and mild hyperinsulinemia, which decreased the liver insulin receptor abundance of the mother but increased that of the fetus. Streptozotocin-induced maternal diabetes resulted in maternal and fetal hyperglycemia with hypoinsulinemia (or near-normal insulin levels) and increased maternal and fetal liver insulin receptors. The maternal and fetal brain insulin receptor abundance failed to change at high or low insulin concentrations. Similarly long-term insulin (5 μg/ml) exposure failed to alter cultured neuronal cell insulin receptors as well. We conclude that perturbations in maternal and fetal circulating insulin concentrations fail to affect the brain insulin receptors. This protective phenomenon may be critical in maintaining the normal neuronal energy balance and functioning during certain disease states such as diabetes mellitus and hyperinsulinism. (AM J OBSTET GYNECOL 1990;163:1350-6.)

Key words: Brain, insulin, receptor, fetal, down-regulation

Diabetes in pregnancy results in various metabolic changes that adversely affect both the mother and the fetus. Type I diabetes results in low insulin concentrations with concomitant hyperglycemia,[1] while type II diabetes is an insulin-resistant state associated with high insulin concentrations and hyperglycemia.[1] In both types maternal hyperglycemia is reflected in the fetus, causing hyperinsulinemia, a state that is responsible for potentiating fetal growth in the third trimester and causing macrosomia and organomegaly.[2] To support the fact that insulin is a fetal growth promoter, there are states of fetal hypoinsulinemia such as pancreatic agenesis or transient diabetes of the newborn, which cause hyperglycemia and intrauterine growth retardation.[3] Recent evidence in various animal species,[4, 5] including adult man,[6] demonstrates that circulating insulin crosses the blood-brain barrier and gains access to the brain via the cerebrospinal fluid. In vitro insulin has been demonstrated to potentiate the growth of both neurons[7] and glial cells,[8] raising the question of whether a similar biologic (hormonal) action occurs in vivo.

From the Division of Neonatology, Department of Pediatrics, St. Louis University, Cardinal Glennon Children's Hospital, and the Pediatric Research Institute.
Part of this work was supported by the Fleur de Lis Foundation and Monsanto-Searle Research Funds, St. Louis, Missouri.
Received for publication August 23, 1989; revised March 21, 1990; accepted June 15, 1990.
Reprint requests: S. Devaskar, MD, 1465 S. Grand Blvd., St. Louis, MO 63104.
6/1/23230

Insulin receptors mediate insulin action within a cell.[9] Down- or up-regulation of insulin receptors is associated with concomitant metabolic changes.[1] Long-term exposure of various adult tissues to high or low insulin concentrations results in either down- or up-regulation of the insulin receptors along with altered metabolism.[10, 11] Fetal insulin receptors, on the other hand, are resistant and have been demonstrated to up regulate in response to high insulin concentrations,[12, 13] causing a blunting effect of the hormone on fetal metabolism.[14]

Despite the demonstration of circulating insulin gaining access to the cerebrospinal fluid in various animal models,[4-6] the non-pregnant adult brain insulin receptor has expressed unique resistance to perturbations in the circulating insulin concentrations.[15, 16] Short-term administration of insulin both systemically and intracranially failed to down regulate the fetal rabbit brain insulin receptor.[17] However, in vitro, long-term rather than short-term exposure to insulin resulted in a down-regulation of chick embryo neuronal insulin receptors.[18] If a similar phenomenon occurred in the in vivo situation, long-term exposure of the maternal and fetal brain to high insulin concentrations would result in insulin receptor changes, which in turn could alter brain metabolism and growth. This is of concern, especially during the fetal stages of development, when neurogenesis is actively taking place.[19] We therefore undertook a study to investigate this possibility in the in vivo situation employing the rat as an animal model.

We produced two models: (1) a model in which both the mother and the fetus were chronically exposed to hyperinsulinemia and (2) a model in which both of them were exposed to hypoinsulinemia, and then the effects on the maternal and fetal brain and liver cross-linked insulin receptor abundance were studied.

Methods

Animals. The guidelines for the care and use of all animals as approved by St. Louis University were followed.

In vivo experiments. Timed pregnant Sprague-Dawley rats (term ~21 days) were used for these studies. Maternal and fetal hyperinsulinemia, together with high glucose concentrations, was produced in vivo by infusing glucose ($n = 5$) (controls, $n = 4$, received sterile distilled water infusions) into the mothers (for 5 days; 24% of gestation). Low insulin concentrations with hyperglycemia were produced by streptozotocin treatment ($n = 4$) (controls, $n = 5$, received saline solution injections), resulting in 6 days of hyperglycemia (28% of gestation), as described previously.[20] Briefly 13-day-pregnant rats were administered pentobarbital for anesthesia and underwent cannulation of the external jugular vein. At 24 hours after the surgical procedure, maternal and fetal hyperglycemia with hyperinsulinemia was produced in some rats by administration of a 30% glucose-in-water infusion at a rate of 17 mg/kg/min. The controls, on the other hand, received a sterile distilled water infusion at a similar rate until 18 to 20 days of gestation. Maternal diabetes was induced in 12-day-pregnant rats by intraperitoneal injections of streptozotocin (60 mg/kg), while the controls received saline solution injections alone. Maternal tail vein glucose values were monitored by Chemstrip bG (Boehringer-Mannheim, Indianapolis), 48 hours (14 to 15 days' gestation) after the initiation of the infusions or the streptozotocin treatment. Before removal of the maternal and fetal organs, plasma was obtained from mothers and fetuses (at 18 to 20 days' gestation). The glucose concentrations were determined by the glucose oxidase method of Yellow Springs Instrument Co., Yellow Springs, Ohio,[17] and the insulin concentrations were measured by a radioimmunoassay.[17] Statistical differences between the two groups (treatment versus respective control) were determined by Student's t test. Maternal and fetal brains and livers were obtained at 18 to 20 days' gestation and crude membranes were prepared by differential centrifugation.[21] Membrane protein content was determined by the Bio-Rad assay (Bio-Rad Laboratories, Richmond, Calif.).[22]

Brain cell preparation. Neuron-enriched cultures[23] were prepared from fetal-neonatal rabbits and characterized as previously described.[23] The rabbit was chosen for in vitro studies as the cellular yield is higher and we have extensively characterized the rabbit neuronal cells by immunocytochemistry.[23] The purity of the neuronal cultures was determined to be ~85% by positive staining of cells for the neuron-specific enolase enzyme,[23] while glial contamination was determined by the presence of glial fibrillary acidic protein staining of cells.[23] The neuron-enriched cultures were maintained in 10 ml of a serum-free defined medium, which contained a high D-glucose concentration[19] (3 mg/ml) either with or without porcine insulin (Eli Lilly & Co., Indianapolis) (5 μg/ml) for a period of 5 days. The cells were then washed extensively and scraped with a rubber policeman before homogenization and centrifugation.[24] Protein content in the membrane pellet was determined by the Bio-Rad dye-binding assay.[22]

Iodine 125–insulin–binding assay. Porcine insulin (Eli Lilly & Co.) was iodinated to a specific activity of 100 to 150 μCi and used as a ligand.[25] A ^{125}I-insulin–binding assay with 0.5 mg of membrane protein was carried out as previously described by us.[20] Briefly, ^{125}I-insulin was incubated at 4° C in the presence (nonspecific) or absence of 1×10^{-6} mol/L native insulin along with the membrane and 1% bovine serum albumin in a final volume of 0.5 ml of Hepes buffer, pH 7.8. The bound fraction was separated from the free fraction by centrifugation. The ^{125}I-insulin–bound receptor was cross-linked with 0.1 mmol/L disuccinimidyl suberate for 16 minutes at 4° C.[26] The cross-linked ^{125}I-insulin–receptor complex was solubulized in Laemmli's buffer before the samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[27] Protein separation was accomplished by use of a discontinuous 5% sodium dodecyl sulfate–polyacrylamide gel system at a constant current of 30 mA per gel. The protein bands were then stained with Coomassie blue, dried, and subjected to autoradiography for at least 48 hours.[27] The autoradiographs were scanned with a Bio-Rad densitometer and the density-area of the band was calculated. The band density-area changes were taken to represent the changes in receptor abundance alone, as a change in receptor affinity has previously not been observed by Scatchard analysis in response to insulin perturbations.[15-17]

Results

A quick screening of maternal glucose concentrations by the Chemstrip bG method at 14 to 15 days' gestation revealed that the maternal glucose infusions (mean ± SEM, 117.5 ± 10.48 vs control of 71.88 ± 4.30 mg/dl; $p < 0.01$) and streptozotocin treatment (190 ± 32 vs a control of 105 ± 5 mg/dl; $p < 0.05$) caused maternal hyperglycemia by 48 hours. The discrepancy between the glucose values of the two control groups is secondary to a relative dilution of the glucose concentrations in the former group by the sterile water infusions.

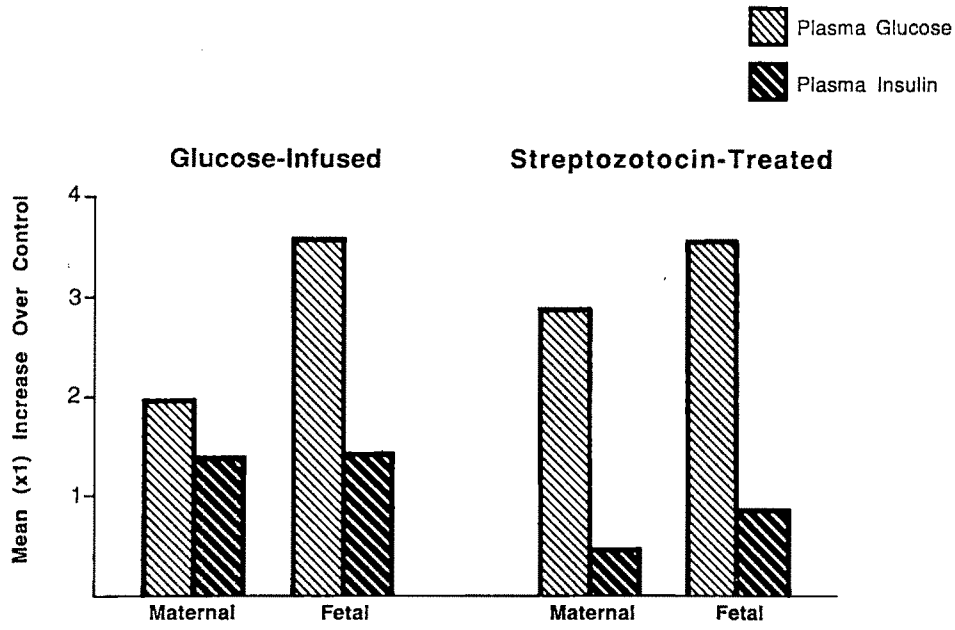


Fig. 1. Plasma glucose and insulin concentrations. Mean increase or decrease in maternal and fetal plasma glucose and insulin concentrations over the corresponding control that was standardized to a value of 1 is shown.

These infusions led to relatively lower control glucose values when compared with those of the control rats, which merely received intraperitoneal injections of normal saline solution.

Fig. 1 demonstrates the mean increase or decrease of plasma glucose and insulin concentrations (at 18 to 20 days' gestation) over a corresponding control that was standardized to 1 for comparison purposes. This standardization was necessary as again the two control values for glucose and insulin were different from each other. Glucose infusions produced a twofold to threefold increase in the maternal (369 ± 79 mg/dl vs a control of 187 ± 7 mg/dl; $p < 0.05$) and a 3.6-fold increase in the fetal (155 ± 25 vs a control of 43 ± 3 mg/dl; $p < 0.01$) plasma glucose levels, respectively. This increase in the plasma glucose level was associated with a 1.5-fold increase in maternal and fetal plasma insulin concentrations. Streptozotocin treatment, on the other hand, while increasing the maternal (317 ± 11 mg/dl; $p < 0.01$) and fetal (214 ± 6 mg/dl; $p < 0.01$) plasma glucose concentrations threefold and 3.6-fold over control (maternal, 109 ± 3 mg/dl; fetal, 60 ± 12 mg/dl), decreased the maternal insulin concentrations to half and the fetal levels to 0.85 of control.

Figs. 2 and 3 represent autoradiographs of the glucose-infused and streptozotocin treatment experiments. Maternal and fetal liver insulin receptor α-subunit (the subunit that binds the hormone) demonstrates a molecular mass of ~135,000, while the maternal and fetal brain receptor is only ~125,000 as observed previously by us.[20] The insulin receptor capacity as determined by the band density-area is decreased in the maternal liver and increased in the fetal liver in response to high circulating insulin concentrations (glucose-infused), while the brain insulin receptor band density-area remained unchanged. In the streptozocin treatment studies hypoinsulinemia in the mother resulted in an increase in the maternal liver insulin receptor band density-area, yet low but normal fetal insulin levels increased the fetal liver insulin receptor band density-area. Again the brain insulin receptor density remained unchanged.

Fig. 4 demonstrates the neuronal insulin receptor. As reported previously, the neuronal insulin receptor, similar to the whole brain, has a molecular mass of ~125,000.[20] The neuronal insulin receptor band density-area akin to that of the whole brain did not change as a secondary result of insulin exposure.

Comment

We have presented evidence to support the fact that long-term maternal and fetal exposure to insulin both in vivo and in vitro, while altering the insulin receptor density-capacity in the liver, fails to do so in the brain, specifically in the neurons. This observation is similar to previously reported results in the adult nonpregnant rat[15, 16] and rabbit fetus in association with acute episodes of hyperinsulinemia.[17] Although a

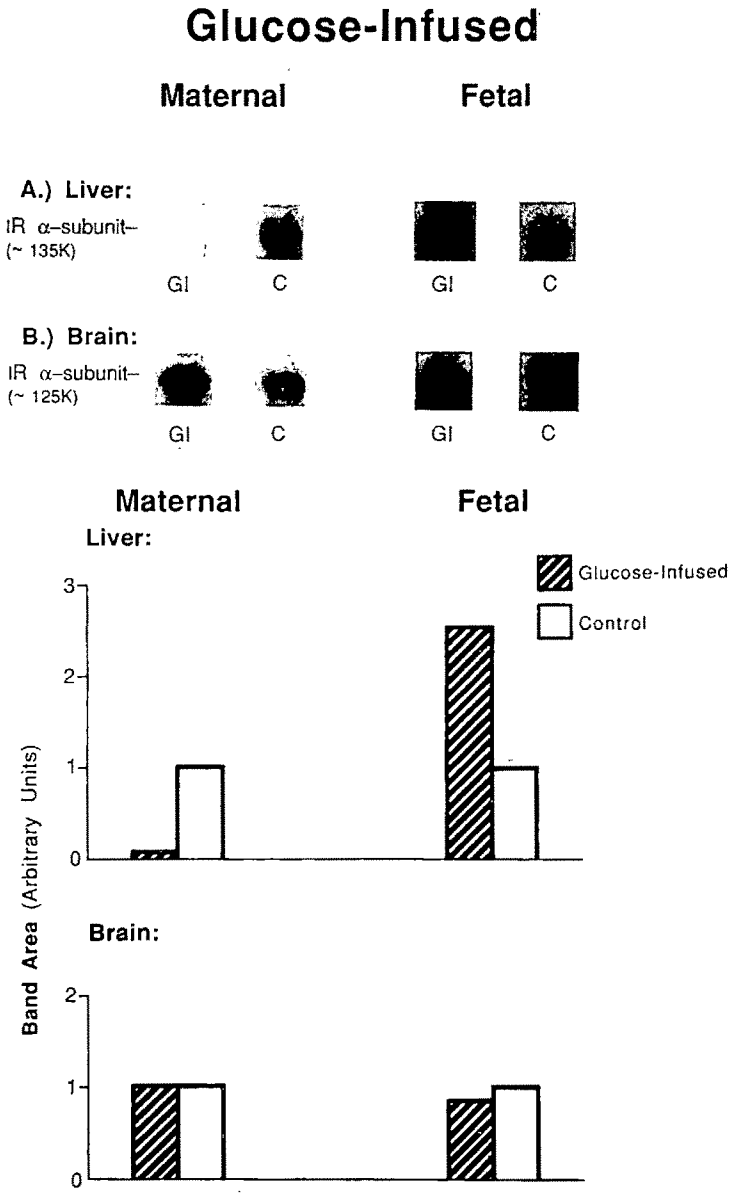


Fig. 2. Insulin receptor (α-subunit) in glucose infusion studies. *Top panel* demonstrates autoradiographs with insulin receptor bands. **A**, Maternal and fetal liver insulin receptors in glucose-infused *(GI)* and control *(C)* groups are seen. **B**, Maternal and fetal brain insulin receptors in the two groups are seen. *Bottom panel* depicts corresponding densitometric assessment of band area. Control is standardized to 1.

down-regulation of neuronal insulin receptors was observed in avian embryonic neurons in culture,[18] our studies demonstrate that this insulin responsiveness of the brain insulin receptor does not persist but disappears during late mammalian fetal life. This may be secondary to species differences but more important may be due to the difference in the stages of maturation. The chick embryo represents a rather early stage of development while in the rat and rabbit a later stage, i.e., the third-trimester fetus, was specifically studied. We chose this developmental phase because the growth-promoting effect of insulin has been reported mainly during this period in mammalian tissues including the brain.[7, 8] However, the presence of insulin-induced down-regulation of the early embryonic neuronal insulin receptor may alter the process of neurogenesis and partly mediate the development of neural defects observed in fetuses of diabetic pregnancies.[1]

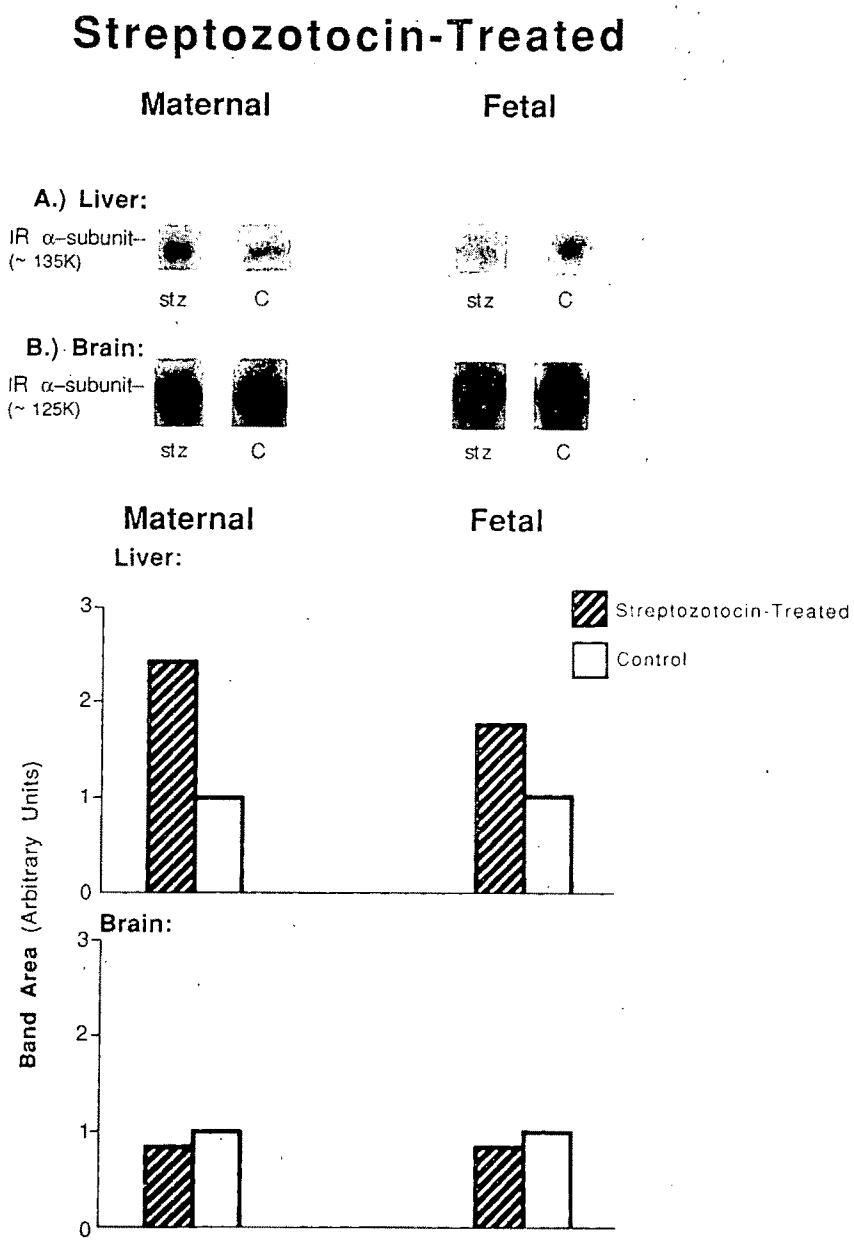


Fig. 3. Insulin receptor (α-subunit) in streptozotocin-treatment studies. *Top panel* demonstrates autoradiographs with insulin receptor bands. **A**, Maternal and fetal liver insulin receptors in streptozotocin *(stz)*—treated and control *(C)* groups are seen. **B**, Maternal and fetal brain insulin receptors in the two groups are seen. *Bottom panel* depicts corresponding densitometric assessment of band area. Control is standardized to 1.

As previously described, the maternal liver insulin receptor was down regulated in response to high insulin concentrations and up regulated as a result of low insulin concentrations.[10, 11] The fetal liver insulin receptor number, on the other hand, was up regulated in both states, i.e., the hyperinsulinemic situation[12, 13] and in association with streptozotocin-induced maternal diabetes.[28] This phenomenon may be due to decreased fluidity of the fetal liver membranes as a result of maternal diabetes,[28] thus resulting in an inability to down regulate surface receptors. There may be other explanations for this phenomenon that have not so far been described, whereby the fetal liver insulin receptor is resistant to insulin-induced down regulation. However, the explanation does not lie in a difference in either the glycosylation[20] or the autophosphorylation[29] of fetal insulin receptors when compared with that of the adult counterpart.

Circulating insulin has been demonstrated to gain access to the cerebrospinal fluid in the adult rat.[4] Al-

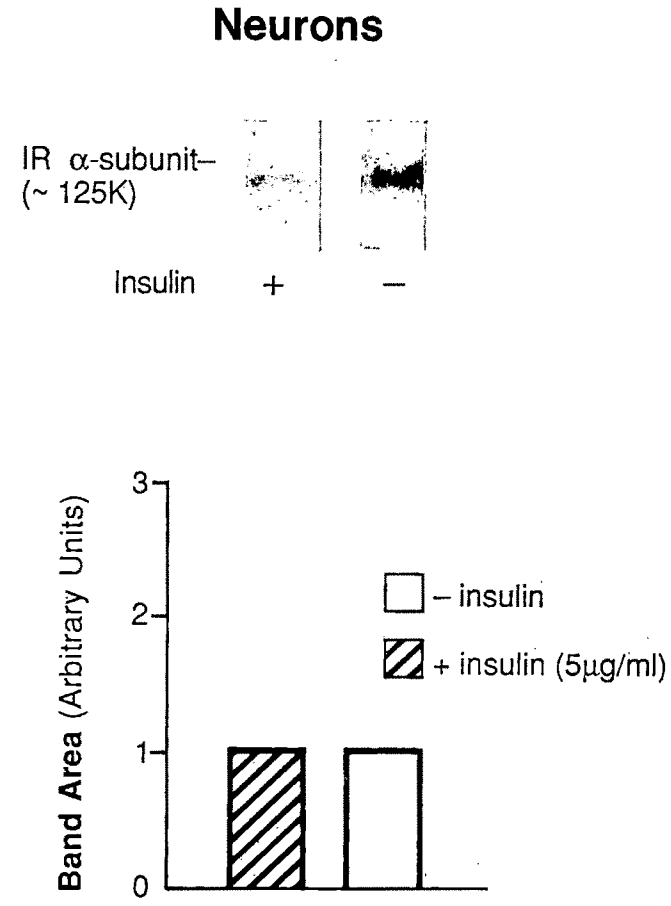


Fig. 4. Neuronal insulin receptors. *Top panel* depicts neuronal insulin receptor band in presence (+) and absence (−) of insulin (total of 50 μg). *Bottom panel* demonstrates corresponding densitometric assessment of band area. Control (−) is standardized to 1.

though short-term perturbations in plasma insulin concentrations are not reflected in the cerebrospinal fluid, long-term changes in the circulating levels of the hormone are reflected in the cerebrospinal fluid in an integrated fashion.[4] Additionally, in the newborn rabbit, labeled insulin injected into the carotid artery gained access to the brain parenchyma.[30] Thus in both our models with hyperinsulinemia and hypoinsulinemia a period of 5 to 6 days is more than adequate to attain parallel changes within the cerebrospinal fluid (and possibly brain parenchyma). Yet long-term exposure of the brain to changes in insulin concentrations failed to alter (down regulate) both maternal and fetal brain insulin receptors. More interesting, however, is the fact that the neuronal cell insulin receptor exposed directly to insulin is resistant as well.

These findings are consistent with the functional significance of these receptors; the liver insulin receptor mediates the traditional effects of insulin such as enhancing the conversion of glucose to glycogen and thereby influencing the glucose homeostasis.[14] The neuronal insulin receptor, on the other hand, mediates unconventional insulin effects such as augmentation of neurite growth,[7] neuronal differentiation,[31] and modulation of neurotransmission[32] while playing no role in neuronal glucose metabolism.[32] An inability of the neuronal insulin receptor to down regulate in response to insulin suggests a protective phenomenon by the cell against perturbations in cerebrospinal fluid insulin concentrations. Recently, we demonstrated that the neuronal cell synthesizes insulin (or an insulin-like substance) as an extrapancreatic source of the hormone.[23] Whether endogenous production of the hormone causes a cellular resistance that results in the inability of the exogenous hormone to down regulate its specific receptor remains unknown.

The physiologic significance of our observation, however, is that in chronically hyperinsulinemic or hypoinsulinemic third-trimester fetuses, while the liver metabolism may be (adversely) altered because of down regulation or up regulation of the insulin receptor, the neuronal cell metabolism is relatively protected, thus allowing neuronal energy balance and functioning to proceed. Whether neuronal growth and differentiation

continue normally in the face of altered circulating insulin concentrations during the first and second trimesters is an issue that requires further investigation in the in vivo mammalian model.

REFERENCES

1. DeFronzo RA, Simonson D, Ferrannini E. Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1982;23:313-20.
2. Devaskar S, Sperling MA. Infants of diabetic mothers. In: Kelly VC, ed. Practice of pediatrics. New York: Harper & Row, 1982-83 vol 24:1-12.
3. Sperling MA, Devaskar S. Transient diabetes of the newborn. In: Kelly VC, ed. Practice of pediatrics. New York: Harper & Row, 1982-83 vol 23:1-3.
4. Stein LJ, Dorsa DM, Baskin DG, et al. Immunoreactive insulin levels are elevated in the cerebrospinal fluid of genetically obese Zucker rats. Endocrinology 1983;113: 2299-301.
5. Woods SC, Porte D Jr. Relationship between plasma and cerebrospinal fluid insulin levels of dogs. Am J Physiol 1977;233:E331-4.
6. Wallum BJ, Taborsky GJ Jr, Porte D Jr, et al. Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. J Clin Endocrinol Metab 1987;64: 190-4.
7. Aizenmann Y, Weischel ME Jr, deVellis J. Changes in insulin and transferrin requirements of pure brain neuronal cultures during embryonic development. Proc Natl Acad Sci USA 1986;83:2263-6.
8. Saneto RP, deVellis J. Hormonal regulation of the proliferation and differentiation of astrocytes and oligodendrocytes in primary culture. In: Schubert D, eds. Developmental biology of cultured nerve, muscle and glia. Somerset, New Jersey: John Wiley, 1984 vol 4:125-67.
9. Czech MP. The nature and regulation of the insulin receptor: structure and function. Ann Rev Physiol 1985;47: 357-81.
10. Olefsky JM. Decreased insulin binding to adipocytes and circulating monocytes in obesity. J Clin Invest 1976;57: 1165-72.
11. Davidson MB, Kaplan SA. Increased insulin binding to hepatic plasma membranes from diabetic rats: normalization by insulin therapy. J Clin Invest 1977;59:22-30.
12. Neufeld ND, Corbo L, Kaplan SA. Plasma membrane insulin receptors in fetal rabbit lung. Pediatr Res 1981; 51:1081-5.
13. Neufeld ND, Kaplan SA, Lippe BM, Scott M. Increased monocyte receptor binding of ^{125}I-insulin in infants of gestational diabetic mothers. J Clin Endocrinol Metab 1978;47:590-5.
14. Sinha MK, Miller JD, Sperling MA, Suchy FJ, Ganguli S. Possible dissociation between insulin binding and insulin action in isolated fetal rat hepatocytes. Diabetes 1984;33: 864-71.
15. Havrankova J, Roth J, Brownstein MJ. Concentrations of insulin and insulin receptors in brain are independent of peripheral insulin levels: studies of obese and streptozotocin-treated rodents. J Clin Invest 1979;64:636-42.
16. Pacold ST, Blackard WG. Central nervous system insulin receptors in normal and diabetic rats. Endocrinology 1979;105:1452-7.
17. Devaskar SU, Karycki L, Devaskar UP. Varying brain insulin concentrations differentially regulate the fetal brain insulin receptor. Biochem Biophys Res Commun 1986; 136:208-19.
18. Heidenrich KA, deVeillis J, Gilmore PR. Functional properties of the subtype of insulin receptor found on neurons. J Neurochemistry 1988;51:878-87.
19. Bottenstein JE. Growth and differentiation of neural cells in defined media. In: Bottenstein JE, Sato G, eds. Cell culture of neurosciences. New York: Plenum Press, 1985 vol 1:3-43.
20. Devaskar S, Holtzclaw L, Sadiq F. The heterogeneity of the developing brain insulin receptor. Pediatr Res 1988; 24:683-92.
21. Lowe W Jr, LeRoith D. Insulin receptors from guinea pig liver and brain: structural and functional studies. Endocrinology 1986;118:1669-77.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72: 248-54.
23. Schechter R, Holtzclaw L, Sadiq F, Kahn A, Devaskar S. Insulin synthesis by isolated rabbit neurons. Endocrinology 1988;123:505-13.
24. Lowe WL, Boyd FT Jr, Clarke DW, Raizada MK, Hart C, LeRoith D. Development of brain insulin receptors: structural and functional studies of insulin receptors from whole brain and primary cell cultures. Endocrinology 1986;119:25-35.
25. Sodoyez JC, Goffaux FS, Goff MM, Zimmerman AG, Arquilla ER. (^{127}I)- or carrier-free (^{125}I) monoiodoinsulin: preparation, physical, immunological and biological properties and susceptibility to "insulinase" degradation. J Biol Chem 1975;250:4268-77.
26. Taylor SI, Samuels B, Roth J, et al. Decreased insulin binding in cultivated lymphocytes from two patients with extreme insulin resistance. J Clin Endocrinol Metab 1982; 54:919-30.
27. Laemmli UK. Cleavage of structural proteins during assembly of the head bacteriophage T4. Nature 1970;227: 680-5.
28. Neufeld ND, Corbo LM. Insulin receptor development in normal and diabetic pregnancies. Diabetes 1986;35: 1020-6.
29. Sinha MK, Jenquin M. Subunit structure, autophosphorylation and tyrosine-specific protein kinase activity of hepatic insulin receptors in fetal, neonatal and adult rats. Diabetes 1987;36:1161-6.
30. Duffy KR, Partridge WM. Blood-brain barrier transcytosis of insulin in developing rabbits. Brain 1987;420: 32-8.
31. Puro DG, Agardh E. Insulin-mediated regulation of neuronal maturation. Science 1984;225:1170-2.
32. Boyd FT Jr, Clarke DW, Muther TF, Raizada MK. Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. J Biol Chem 1985;260:15880-5.

Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb

René P. Paulick, MD,[a] Rebecka L. Meyers, MD,[b] Colin D. Rudolph, MD, PhD,[c] and Abraham M. Rudolph, MD[c]

San Francisco, California

Evidence is accumulating that prostaglandins affect the tone of the ductus venosus. Therefore prostaglandins might alter the distribution of umbilical venous return between the ductus venosus and liver. We have examined the effect of indomethacin and prostaglandin E_1 on the vascular resistance of the umbilical-placental circulation, ductus venosus, and liver in 14 chronically instrumented fetal lambs. We found that indomethacin caused a nearly twofold increase in the vascular resistance of both the ductus venosus and liver. Prostaglandin E_1, administered 70 minutes after indomethacin at a dose of 0.41 ± 0.09 (SEM) μg/min per kilogram of fetal weight, decreased umbilical-placental blood flow, increased the vascular resistance of the umbilical arteries and placenta, and reversed the elevation of the vascular resistances of the ductus venosus and liver. Because both indomethacin and prostaglandin E_1 affected the resistances of the ductus venosus and liver to the same extent, it is unlikely that prostaglandins of the E series mediate the change in distribution of umbilical venous return between the ductus venosus and liver during fetal stress. (Am J Obstet Gynecol 1990;163:1357-63.)

Key words: Blood flow, blood pressure, radioactive microspheres, prostaglandins, indomethacin

During fetal development about 40% of the combined ventricular output is distributed to the placenta. Blood returns to the fetus through the umbilical veins to the portal sinus; it may then pass through the hepatic microcirculation or bypass the liver through the ductus venosus. Normally, about half the umbilical venous return passes through the ductus venosus and half through the liver.[1] These proportions change during fetal stress, particularly when umbilical blood flow falls, with a greater proportion of umbilical venous return passing through the ductus venosus.[2-5] The factors that determine the distribution of blood flow between the ductus venosus and liver have not been defined but may involve constriction or relaxation of the ductus venosus.

Evidence is accumulating that prostaglandins affect the tone of the ductus venosus. With the use of rings of ductus venosus, Adeagbo et al.[6,7] showed constriction with indomethacin, a prostaglandin synthesis inhibitor, and relaxation with prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2). A preliminary report of an in vivo study in fetal lambs supported these results.[8] In addition, prostaglandin E_1 (PGE_1), a synthetic analog of PGE_2, has been shown to reopen the closed ductus venosus in newborn lambs.[9] However, none of these studies has measured the actual changes in vascular resistance across the ductus venosus and liver in response to indomethacin and PGE_1.

To determine the influence of indomethacin and PGE_1 on the vascular resistance of the ductus venosus, liver, umbilical veins, umbilical arteries, and placenta in fetal lambs, we measured blood pressure in the fetal descending aorta, extraabdominal umbilical vein, portal sinus, hepatic vein, and inferior vena cava; we also measured blood flow with the microsphere technique. From these data we calculated changes in the vascular resistance of each component of the umbilical-placental circulation.

Material and methods

Surgery and experiments were performed according to the guidelines for the care and use of animals approved by the Committee on Animal Research, University of California, San Francisco. Fourteen mixed-breed Western ewes with time-dated gestation of 122 to 137 days at the time of study (term, 145 days) were fasted 24 to 48 hours before spinal anesthesia was induced with 2 to 4 ml of 1% tetracaine hydrochloride (Ponto-

From the Cardiovascular Research Institute and the Departments of Pediatrics,[c] Surgery,[b] and Obstetrics, Gynecology and Reproductive Sciences,[a] University of California, San Francisco.
Supported by National Heart, Lung, and Blood Institute Program Project Grant HL-24056 and a Deutsche Forschungsgemeinschaft Research Fellowship (DFG Pa 342/1-1) awarded to Dr. Paulick.
Received for publication January 9, 1990; revised May 16, 1990; accepted June 15, 1990.
Reprint requests: A. M. Rudolph, MD, Box 0544, HSE 1403, University of California, San Francisco, San Francisco, CA 94143.
6/1/23234

caine, Winthrop Labs., New York) and they were placed supine on the operating table. Polyvinyl catheters (inner diameter, 0.05 inches) were inserted into pedal branches of a maternal femoral artery and vein and advanced to the descending aorta and inferior vena cava. An intravenous infusion of 0.9% sodium chloride (500 to 1000 ml) was administered to the ewe throughout the procedure. Ketamine hydrochloride, 50 to 100 mg, was injected intravenously as necessary to maintain sedation. With the use of strictly aseptic techniques, the ewe's abdomen was opened in the midline. Anesthesia for the placement of all fetal catheters was induced locally with 0.5% lidocaine hydrochloride and systemically with 15 to 20 mg of intramuscular ketamine. The fetal hind limbs were exposed through a small uterine incision, and polyvinyl catheters (inner diameter, 0.03 inches) inserted in each leg via pedal branches into the infrarenal descending aorta. Through a pedal branch of the femoral vein, a 3.5 F multiple side-hole catheter was advanced centrally so that the tip was at the level of the diaphragm where the ductus venosus and hepatic veins enter the inferior vena cava. Two 3.5 F multiple side-hole catheters were inserted via cotyledonary tributaries into the extraabdominal umbilical vein. The tips of the umbilical vein catheters used for pressure measurements were on average 27 cm distal to the umbilical ring. An amniotic catheter (inner diameter, 0.05 inches) was inserted to lie adjacent to the fetal trunk, and the uterine incision was closed.

A catheter was placed in the portal sinus in eight fetuses. Through a separate uterine incision, the fetal abdomen was exposed and opened and a loop of small intestine isolated. A 3.5 F multiple side-hole catheter was inserted into a mesenteric venous tributary and advanced toward the hilum of the liver until blood withdrawn from the catheter showed increased oxygenation, indicating mixture with umbilical venous blood. When the oxygen saturation of the blood in this catheter matched that of blood in the umbilical vein, the catheter was drawn back slowly until a sharp drop in oxygen saturation occurred. From this point the catheter was then advanced proximally 1 cm. With this approach the catheter tip was reliably located in the portal sinus.

In six fetuses a catheter was placed in either the left ($n = 4$) or right ($n = 2$) hepatic vein. The method has been previously described in detail.[10] Briefly, through a right thoracotomy, the inferior vena cava was punctured through a purse-string suture and a 3.5 F multiple side-hole catheter was advanced ≈3 cm inferiorly into a hepatic vein. Catheter placement in the hepatic vein was confirmed by comparing the oxygen saturation of blood withdrawn from this catheter with that of blood in the umbilical vein and inferior vena cava.

The fetal and uterine incisions were closed, and amniotic fluid lost during surgery was replaced with warm 0.9% sodium chloride. All catheters were filled with heparin (1000 IU/ml) and plugged. The catheters were then brought subcutaneously to the ewe's flank where they were exteriorized and stored in a vinyl pouch. On the day of surgery and each day afterward, the ewe received 2 million IU of penicillin G and 100 mg of gentamicin, half intravenously and half into the amniotic cavity. Vascular catheters were flushed daily with heparin. The position of all catheters was confirmed at dissection.

Fourteen studies, one of each fetus, were performed 2 to 6 days after surgery. Fetal descending aortic, amniotic, and venous pressure levels (extraabdominal umbilical vein, portal sinus, hepatic vein, and inferior vena cava) were measured with carefully calibrated pressure transducers (Statham P23Db, Statham Instruments, Oxnard, Calif.). Pressure levels were recorded continuously on a direct writing recorder (Beckman R612, Beckman Instruments, Palo Alto). Baseline pressure levels were recorded for 2 to 3 hours to obtain steady state conditions, minimize zero drifts, and make calibration adjustments. To eliminate errors as a result of possible zero drift in different transducers, all venous pressure levels were recorded with the same transducer with a ramp of Luer-Lok stopcocks (Vygon, Ecouen, France). Each "set" of venous pressures was obtained within a period of 45 to 60 seconds, and pressure levels were accepted as valid only when amniotic and arterial pressures remained constant during this time. Throughout the experiment, zero levels were checked every 10 to 15 minutes and did not vary >1 mm Hg. Fetal heart rate was measured with a cardiotachometer triggered by the arterial pulse pressure. Amniotic pressure was used as the zero pressure reference.

Control blood samples were withdrawn from the fetal descending aorta. Blood gas and pH levels were measured with a blood gas analyzer (Corning 175, Medfield, Mass.). Hemoglobin concentrations and blood oxygen saturation were measured photometrically in duplicate (Hemoximeter OSM 2, Radiometer, Copenhagen, Denmark). A bolus of approximately 2 million (15.0 ± 0.3 SD) μm microspheres labeled with different isotopes randomly selected from cobalt 57, chromium 51, gadolinium 153, indium 114, manganese 54, niobium 95, tin 113, strontium 85, and zinc 65 (3M Company, St. Paul, and New England Nuclear, Boston) was then injected into the umbilical vein. A reference arterial blood sample was withdrawn from the descending aorta at ≈3.6 ml/min, beginning 10 to 15 seconds before the microsphere injection and lasting 75 to 90 seconds. The volume of blood withdrawn was replaced with an equal volume of fetal donor blood or, if this was not available, with maternal blood. A dose of 0.33 ± 0.07 (mean ± SEM) mg/kg fetal weight indomethacin (Merck, Sharp & Dohme, West Point, Pa.) was then injected into

the umbilical vein. Sixty minutes later, venous pressure levels and blood samples from the descending aorta were obtained and microspheres labeled with different radionuclides were injected into the umbilical vein. After 10 minutes (i.e., 70 minutes after the injection of indomethacin) PGE_1 (Upjohn, Kalamazoo, Mich.) was infused diluted in 0.9% sodium chloride with a Harvard pump at a dose of 0.41 ± 0.09 (SEM) μg/min per kilogram of fetal weight into the umbilical vein for 20 minutes; 15 minutes after beginning the PGE_1 infusion, venous pressure levels and blood samples were obtained and a third set of randomly chosen microspheres was injected into the umbilical vein.

At the end of the study the ewe was killed with an overdose of pentobarbital sodium. The uterus and individual fetal organs were dissected and weighed. All organs were placed in formalin, incinerated at 350° C, ground into a coarse powder, and placed into plastic vials to a uniform height of 3 cm.[11] The vials were counted in a well-type γ-scintillation counter and analyzed with a multichannel (1024) pulse height analyzer (Norland, Ford Atkinson, Wis.). The activity of each isotope in each organ was determined from an analysis method of least squares described previously.[12]

Microspheres injected into an umbilical vein are either trapped in the liver or passed through the ductus venosus to the inferior vena cava. Thus the proportion of umbilical venous return distributed to the liver can be calculated from the counts in the liver divided by the total counts recovered from the fetus and placenta.[1] Subtraction of this proportion from 1 yields the proportion of umbilical venous return that passes through the ductus venosus. Adequate mixing of microspheres in umbilical venous blood has been shown in a previous study in which microspheres labeled with different radionuclides were simultaneously injected into different branches of the umbilical veins.[2] These studies show that the distribution of microspheres injected into one branch of the umbilical vein represents the pattern of distribution of all the umbilical venous blood.

Except for the liver, blood flows to the lower body organs and placenta were calculated from the counts in the organ, divided by the counts in the reference sample, and miltiplied by the reference sample blood flow.[11-13] Umbilical vein-derived blood flow to the left and right lobes of the liver and through the ductus venosus was calculated by multiplying the umbilical-placental blood flow by the proportion of umbilical venous return distributed to each lobe and through the ductus venosus, respectively. Blood flow to the right lobe of the liver was then calculated by adding portal venous blood flow to the umbilical venous flow distributed to the right liver. Finally, total liver blood flow was calculated by adding that of the left and right lobe.

These calculations are made on the basis of previous studies that have shown that the right lobe of the liver receives almost all the portal venous blood and a contribution of umbilical venous blood.[1] The left lobe of the liver and the ductus venosus receive primarily umbilical venous blood. The hepatic arteries contribute <10% of total liver blood flow in the fetus[1,4]; this was considered negligible. Portal blood flow was calculated as the sum of flows to the spleen, pancreas, and gut. Blood flows are expressed as milliliter per minute per kilogram of fetal weight.

Total resistance to umbilical-placental blood flow was calculated from umbilical-placental blood flow and the pressure difference between the descending aorta and inferior vena cava. With the use of the pressure levels measured in the umbilical vein and portal sinus, this total vascular resistance was subdivided as follows: (1) resistance to umbilical-placental blood flow between the descending aorta and umbilical vein, representing the resistance of the umbilical arteries and placental microvasculature; (2) resistance between the umbilical vein and portal sinus, representing the resistance of the umbilical veins; and (3) resistance between the portal sinus and inferior vena cava, representing the combined resistance of the ductus venosus and liver. Individual resistances of the ductus venosus and the liver were then calculated by dividing the pressure difference between the portal sinus and inferior vena cava by the appropriate blood flow levels. In the six fetuses with a hepatic vein catheter, pressure levels measured simultaneously in the hepatic vein and inferior vena cava were not significantly different throughout the experiments. Therefore we used the inferior vena caval pressure level for the above resistance calculations. Values for vascular resistance are expressed as millimeters of mercury per milliliter per minute per kilogram of fetal weight.

Data analysis was performed with a computerized statistical program (Statview II, Abacus Concepts, Berkeley, Calif.). When a normal distribution was present, data are expressed as mean ± SEM. Comparison between the three experimental periods was made by one-way analysis of variance for repeated measurements. Multiple comparison testing was performed only if the analysis of variance first rejected a multisample hypothesis of equal means with $p < 0.05$. Differences between groups were then analyzed with the Scheffé multiple comparison F test. For the abdominal inferior vena caval blood flow and the resistance of the liver, a skewed distribution was shown by box plots. In these two cases, results are expressed as mean ± SEM after eliminating one extreme outlying value; for multiple comparison testing, we used the Friedman test and analyzed differences between groups with the Wilcoxon test with the Bonferroni adjustment. For all tests, $p < 0.05$ was considered statistically significant.

Table I. Fetal pH, blood gas levels, and hemoglobin concentrations during the control period, 60 minutes after injection of indomethacin, and during a PGE_1 infusion*

Descending aorta	*Control*	*Indomethacin*	*PGE_1 infusion**
pH	7.36 ± 0.01	7.32 ± 0.02	7.30 ± 0.01†
P_{CO_2} (mm Hg)	53.8 ± 1.8	55.2 ± 2.3	56.8 ± 2.1
P_{O_2} (mm Hg)	23.9 ± 1.7	23.5 ± 1.6	21.3 ± 1.3†
Hemoglobin (gm/dl)	9.6 ± 0.4	9.8 ± 0.4	9.4 ± 0.4
Oxygen saturation (%)	45.0 ± 3.1	42.0 ± 3.3	33.3 ± 3.2‡

Data are mean ± SEM in 14 fetal lambs.

*PGE_1 infusion was started 70 minutes after injection of indomethacin; measurements were made 15 minutes after beginning the PGE_1 infusion.

†$p < 0.05$.

‡$p < 0.001$ control vs PGE_1; $p < 0.01$ indomethacin vs PGE_1.

Table II. Blood pressure levels and pressure differences during the control period, 60 minutes after injection of indomethacin, and during a PGE_1 infusion*

	Control	*Indomethacin*	*PGE_1 infusion**
Mean pressure (mm Hg)			
Descending aorta	45.7 ± 0.8	48.3 ± 1.2†	45.4 ± 1.3‡
Extraabdominal umbilical vein§	9.5 ± 0.6	12.3 ± 1.0‖	10.0 ± 0.7‡
Portal sinus¶	4.2 ± 0.5	5.7 ± 0.6‖	4.9 ± 0.6
Hepatic vein#	3.4 ± 0.2	3.6 ± 0.8	3.2 ± 0.4
Inferior vena cava	2.6 ± 0.3	2.9 ± 0.5	3.1 ± 0.4
Pressure difference between (mm Hg)			
Descending aorta and extra-abdominal umbilical vein	36.2 ± 1.0	36.1 ± 1.1	35.4 ± 1.4
Extraabdominal umbilical vein and portal sinus¶	5.5 ± 0.9	6.6 ± 1.1	5.3 ± 0.9
Portal sinus and inferior vena cava¶	1.7 ± 0.2	3.0 ± 0.4‖	1.6 ± 0.2**

Data are mean ± SEM in 14 fetal lambs.

*PGE_1 infusion was started 70 minutes after injection of indomethacin; measurements were made 15 minutes after beginning the PGE_1 infusion.

†$p < 0.05$.

‡$p < 0.05$.

§Mean distance 27 cm from umbilical ring.

‖$p < 0.01$ control vs indomethacin.

¶$n = 8$.

#$n = 6$.

**$p < 0.001$ indomethacin vs PGE_1.

Results

Fetal weight was 3.3 ± 0.2 kg. During the control period, fetal pH, blood gas levels, and hemoglobin concentrations in the descending aorta were within the normal range of values measured in our laboratory (Table I). Indomethacin injection caused no significant changes in these variables. During the PGE_1 infusion, P_{O_2}, oxygen saturation, and pH decreased.

Fetal heart rate was 186 ± 6 beats/min at control, 202 ± 9 beats/min 1 hour after indomethacin, and 184 ± 10 beats/min during the subsequent PGE_1 infusion; these changes were not significant. Indomethacin significantly increased pressures in the descending aorta, extraabdominal umbilical vein (catheter tip on average 27 cm distal to the umbilical ring), and portal sinus, whereas inferior vena caval pressure was not changed (Table II). All pressures returned to control values during the PGE_1 infusion. In the six experiments in which pressures were measured simultaneously in the inferior vena cava and a hepatic vein, there was a small but insignificant pressure difference between these two sites (0.5 ± 0.2 mm Hg) during all experimental periods.

During the control period the difference in pressure between the descending aorta and extraabdominal umbilical vein, which represents the pressure drop across the umbilical arteries and placenta, was 36.2 ± 1.0 mm Hg (Table II). The difference in pressure between the extraabdominal umbilical vein and the portal sinus, which represents the pressure drop along the umbilical veins, averaged 5.5 ± 0.9 mm Hg, and that between the portal sinus and inferior vena cava, which repre-

Table III. Fetal blood flows during the control period, 60 minutes after injection of indomethacin, and during a PGE_1 infusion*

Blood flow (ml/min/kg)	*Control*	*Indomethacin*	*PGE_1 infusion**
Umbilical-placental	216.8 ± 14.7	230.3 ± 25.3	182.6 ± 20.3†
Ductus venosus	103.1 ± 11.1	102.8 ± 12.1	89.4 ± 7.9
Liver‡	162.2 ± 19.5	165.7 ± 29.4	149.3 ± 21.5
Abdominal inferior vena cava§	127.9 ± 14.8	125.9 ± 20.7	146.2 ± 16.5

Data are mean ± SEM in 14 fetal lambs.

*PGE_1 infusion was started 70 minutes after injection of indomethacin; measurements were made 15 minutes after beginning the PGE_1 infusion.

†$p < 0.01$ indomethacin vs PGE_1.

‡Includes the contribution of the portal vein and umbilical vein.

§Represents blood flow to the lower carcass, kidneys, and adrenals.

Table IV. Vascular resistance to umbilical-placental blood flow during the control period, 60 minutes after injection of indomethacin, and during a PGE_1 infusion*

Vascular resistance†	*Control*	*Indomethacin*	*PGE_1 infusion**
Total	0.239 ± 0.026	0.249 ± 0.034	0.291 ± 0.039‡
Umbilical arteries and placenta	0.200 ± 0.021	0.195 ± 0.025	0.243 ± 0.034‡,§
Umbilical veins	0.029 ± 0.006	0.038 ± 0.009	0.036 ± 0.008
Ductus venosus and liver	0.010 ± 0.002	0.017 ± 0.004‖	0.011 ± 0.002
Ductus venosus	0.020 ± 0.003	0.035 ± 0.006‖	0.023 ± 0.004
Liver	0.013 ± 0.003	0.021 ± 0.005‖	0.015 ± 0.004

Data are mean ± SEM in the 8 fetal lambs in which portal sinus pressure was measured.

*PGE_1 infusion was started 70 minutes after injection of indomethacin; measurements were made 15 minutes after beginning the PGE_1 infusion.

†mm Hg/ml/min/kg.

‡$p < 0.05$ control vs PGE_1.

§$p < 0.01$ indomethacin vs PGE_1.

‖$p < 0.05$ control vs indomethacin.

sents the pressure drop across the ductus venosus and liver, was 1.7 ± 0.2 mm Hg. Indomethacin increased the pressure difference between the portal sinus and inferior vena cava significantly by 76%. During the PGE_1 infusion all pressure differences had returned to control values.

Compared with the control period, indomethacin did not change umbilical-placental, hepatic, ductus venosus, or abdominal inferior vena caval blood flow (Table III). Compared with the flows measured after indomethacin, the PGE_1 infusion decreased umbilical-placental blood flow significantly. Neither indomethacin nor PGE_1 changed the proportion of umbilical venous return that passed through the ductus venosus or the left or right lobe of the liver.

During the control period total vascular resistance to umbilical-placental blood flow averaged 0.239 mm Hg/ml per minute per kilogram of fetal weight. Of this total resistance, the umbilical arteries and placenta accounted for 84%, the umbilical veins 12%, and the ductus venosus and liver 4%. Indomethacin increased the combined vascular resistance of the ductus venosus and liver almost twofold (Table IV). The individual resistance of the ductus venosus increased 75% and that of the liver increased 65%. Furthermore, the vascular resistance of the right and left lobes of the liver increased to the same extent. Infusion of PGE_1 completely reversed this indomethacin effect of the vascular resistance of the ductus venosus and liver. However, total resistance to umbilical-placental blood flow increased as a result of an increase in the resistance of the umbilical arteries and placenta.

Comment

The distribution of the umbilical venous return between the ductus venosus and liver is altered during fetal stress; the proportion of umbilical venous blood that passes through the ductus venosus increases, whereas that to the liver decreases.[2-5] However, the mechanism responsible for this change in distribution of umbilical venous return is not known. Adeagbo et al.[6,7] showed in vitro that indomethacin, a prostaglandin synthesis inhibitor, constricted the ductus venosus, whereas both PGE_2 and PGI_2 resulted in relaxation. Blood concentrations of PGE were found to be elevated during chronic fetal hypoxemia.[14] Thus pros-

taglandins could be important in the regulation of the tone of the ductus venosus during fetal development. To examine this hypothesis we studied the effect of indomethacin and PGE_1, a synthetic analog of PGE_2, on the vascular resistance of the ductus venosus and liver in chronically prepared fetal lambs.

Indomethacin did not change umbilical-placental blood flow[15, 16] or the proportion of umbilical venous return distributed to the ductus venosus and liver. Thus ductus venosus and liver blood flows were unchanged. These results conflict with those in a preliminary report that suggested that indomethacin decreases ductus venosus blood flow in fetal lambs, but no data on umbilical-placental or ductus venosus blood flows were provided.[8]

Indomethacin had no effect on the vascular resistance of the umbilical arteries, placental vasculature, or umbilical veins. Indomethacin increased the vascular resistance of the ductus venosus almost twofold; this effect was reversed by PGE_1. Thus the ductus venosus sphincter appears to be under the control of a relaxing prostaglandin in the fetus. According to Coceani,[17] this relaxing prostaglandin is PGE_2 or PGI_2. However, this effect is not specific for the ductus venosus because hepatic vascular resistance increased to about the same extent as that of the ductus venosus after indomethacin. The site of the increase in hepatic vascular resistance has not been delineated. On the basis of anatomic studies, sphincters have been described presinusoidally at the portal venous-sinusoidal junction and postsinusoidally at the sinusoidal-central vein junction and in the hepatic veins.[18, 19] On the basis of physiologic studies, Lautt et al.[20, 21] proposed that the major site of portal vascular resistance resides in small hepatic veins, beyond the sinusoidal-central vein connection. We were not able to detect any constriction in the large hepatic veins, but constriction may occur in small hepatic veins with a diameter of 1 to 2 mm.

The fact that the vascular resistance of both the ductus venosus and liver increases with indomethacin and decreases with PGE_1 indicates that, in the fetus, prostaglandins are important in maintaining a low resistance to umbilical venous and portal venous return. This effect appears to be similar to that of PGE_2 on the ductus arterious.[17] However, because indomethacin and PGE_1 had no differential effect on the ductus venosus and liver, it is evident that prostaglandins of the E series do not mediate the change in distribution of umbilical venous return between the ductus venosus and liver during fetal stress.

The effect of indomethacin on the ductus venosus was not dramatic and ductus venosus blood flow was well maintained. This observation does not support the proposal that the postnatal decrease in PGE blood concentrations[22] would play a significant role in closing the ductus venosus after birth.[17]

McLaughlin et al.,[15] on the basis of physiologic studies, reported that indomethacin results in vasoconstriction of the umbilical-placental circulation. However, this vasoconstrictive effect was observed immediately after indomethacin injection and was sustained for only 10 minutes. Our results show no change in the vascular resistance of the umbilical arteries, placental vasculature, or umbilical veins 60 minutes after indomethacin injection. As was shown by Naden et al.,[23] blood concentrations of PGE_2 do not decrease immediately after indomethacin. The same authors suggested that indomethacin has immediate vasoconstrictive properties that are independent of the inhibition of prostaglandin synthesis. In support of this, our group found that indomethacin produced an immediate rise in arterial blood pressure in fetal lambs pretreated with meclofenamate, a prostaglandin synthesis inhibitor (M. Heymann, personal communication).

As has been observed previously, PGE_1 decreased umbilical-placental blood flow.[24, 25] This fall in blood flow was a result of an increase in the vascular resistance of the umbilical-placental circulation. The site of this increased resistance has not been determined. It was not in the major umbilical veins, and thus must have been either in the umbilical arteries or the placental microvasculature. On the basis of in vitro studies, PGE_1 may cause constriction of the umbilical arteries.[26, 27]

In conclusion, we showed that prostaglandins maintain a modest degree of relaxation of the ductus venosus and hepatic microcirculation during fetal development but are not responsible for the redistribution of umbilical venous return between the ductus venosus and liver during fetal stress.

We gratefully acknowledge the skillful technical assistance of Christine Roman, Roger Chang, Bruce Payne, and Carl McWatters that made the completion of this project possible. We also acknowledge the editorial assistance of Paul Sagan.

REFERENCES

1. Edelstone DI, Rudolph AM, Heymann MA. Liver and ductus venosus blood flows in fetal lambs in utero. Circ Res 1978;42:426-33.
2. Edelstone DI, Rudolph AM, Heymann MA. Effects of hypoxemia and decreasing umbilical blood flow on liver and ductus venosus blood flows in fetal lambs. Am J Physiol 1980;238:H656-63.
3. Ruess ML, Rudolph AM. Distribution and recirculation of umbilical and systemic venous blood flow in fetal lambs during hypoxia. J Dev Physiol 1980;2:71-84.
4. Itskovitz J, La Gamma EF, Rudolph AM. Effects of cord compression on fetal blood flow distribution and O_2 delivery. Am J Physiol 1987;252:H100-9.
5. Itskovitz J, Goetzman B, Rudolph AM. Effects of hem-

orrhage on umbilical venous return and oxygen delivery in fetal lambs. Am J Physiol 1982;242:H543-8.
6. Adeagbo ASO, Coceani F, Olley PM. The response of the lamb ductus venosus to prostaglandins and inhibitors of prostaglandin and thromboxane synthesis. Circ Res 1982; 51:580-6.
7. Adeagbo ASO, Bishai I, Lees J, Olley PM, Coceani F. Evidence for a role of prostaglandin I_2 and thromboxane A_2 in the ductus venosus of the lamb. Can J Physiol Pharmacol 1985;63:1101-5.
8. Sideris EB, Yokochi K, Van Helder T, Coceani F, Olley PM. Effects of indomethacin and prostaglandins E_2, I_2, and D_2 on the fetal circulation. Adv Prostaglandin Thromboxane Leukotriene Res 1983;12:477-82.
9. Morin FC. Prostaglandin E_1 opens the ductus venosus in the newborn lamb. Pediatr Res 1987;21:225-8.
10. Bristow J, Rudolph AM, Itskovitz J. A preparation for studying liver blood flow, oxygen consumption, and metabolism in the fetal lamb in utero. J Dev Physiol 1981;3:255-66.
11. Heymann MA, Payne BD, Hoffman JIE, Rudolph AM. Blood flow measurements with radionuclide-labeled particles. Prog Cardiovasc Dis 1977;20:55-79.
12. Baer RW, Payne BD, Verrier ED, et al. Increased number of myocardial blood flow measurements with radionuclide-labeled microspheres. Am J Physiol 1984; 246:H418-34.
13. Rudolph AM, Heymann MA. The circulation of the fetus in utero. Circ Res 1967;21:163-84.
14. Challis JRG, Hart I, Louis TM, et al. Prostaglandins in the sheep fetus: implications for fetal function. In: Coceani F, Olley PM, ed. Advances in prostaglandin and thromboxane research. New York: Raven Press, 1978: 115-32.
15. McLaughlin MK, Brennan SC, Chez RA. Effects of indomethacin on sheep uteroplacental circulations and sensitivity to angiotensin II. Am J Obstet Gynecol 1978; 132:430-5.
16. Rudolph AM, Heymann MA. Hemodynamic changes induced by blockers of prostaglandin synthesis in the fetal lamb in utero. In: Coceani F, Olley PM, ed. Advances in prostaglandin and thromboxane research. New York: Raven Press, 1978:231-7.
17. Coceani F, Olley PM. The control of cardiovascular shunts in the fetal and perinatal period. Can J Physiol Pharmacol 1988;66:1129-34.
18. Knisely MH, Harding F, Debacker H. Hepatic sphincters; brief summary of present-day knowledge. Science 1957; 125:1023-6.
19. Elias H, Sherrick HC. Morphology of the liver. New York: Academic Press, 1969.
20. Lautt WW, Greenway CV, Legare DJ, Weisman H. Localization of intrahepatic portal vascular resistance. Am J Physiol 1986;251:G375-81.
21. Lautt WW, Greenway CV. Conceptual review of the hepatic vascular bed. Hepatology 1987;7:952-63.
22. Challis JRG, Patrick JE. The production of prostaglandins and thromboxanes in the feto-placental unit and their effects on the developing fetus. Semin Perinatol 1980; 4:23-33.
23. Naden RP, Iliya CA, Arant BS, Gant NF, Rosenfeld CR. Hemodynamic effects of indomethacin in chronically instrumented pregnant sheep. Am J Obstet Gynecol 1985;151:484-94.
24. Berman W, Goodlin R, Heymann MA, Rudolph AM. Effects of pharmacologic agents on umbilical blood flow in fetal lambs in utero. Biol Neonate 1978;33:225-35.
25. Tripp ME, Heymann MA, Rudolph AM. Hemodynamic effects of prostaglandin E_1 on lambs in utero. In: Coceani F, Olley PM, ed. Advances in prostaglandin and thromboxane research. New York: Raven Press, 1978:221-9.
26. Park MK, Rishor C, Dyer DC. Vasoactive actions of prostaglandins and serotonin on isolated human umbilical arteries and veins. Can J Physiol Pharmacol 1972;50: 393-9.
27. Tuvemo T, Strandberg K. Effects and interactions of oxygen and prostaglandins on the tone of the isolated human umbilical artery. Ups J Med Sci 1975;80:131-4.

Correction

In the article by Fong et al., "Fetal thoracic circumference: A prospective cross-sectional study with real-time ultrasound" (Am J Obstet Gynecol 1988;158:1154-60), in the last paragraph on page 1158, the sentences "The ratio chest circumference/abdominal circumference was found to be constant across gestation. A 95% confidence interval for predicting this ratio for a given gestational age is given by 0.91 ± 0.046" should have been "The ratio chest circumference/abdominal circumference was found to be constant across gestation. A 95% confidence interval for predicting this ratio for a given gestational age is given by 0.88 ± 0.13."

LETTERS TO THE EDITORS

Definition of fetal asphyxia

To the Editors: I enjoyed reading the article on umbilical blood acid-base studies in the March 1990 issue (Johnson JWC, Richards DS, Wagaman RA. The case for routine umbilical blood acid-base studies at delivery. AM J OBSTET GYNECOL 1990;162:621-5). I concur with their opinions. I do have one question: How do they define "fetal asphyxia"? They point out that acid-base measurements are superior to Apgar score, fetal heart rate monitoring, neurologic development, and meconium staining in diagnosing asphyxia, but how low does the pH have to be? Below 7.20? Below 7.00? Is a buffer base of <34 mmol/L sufficient? On page 622 they state that fetal asphyxia is "hypoxia and acidosis." I agree. How low does the Po_2 have to be? <22 mm Hg? Is an infant with a Po_2 of 0 automatically suffering from asphyxia? They state that "umbilical blood acid-base studies are the only scientific, objective measure of fetal asphyxia . . ." What is the scientific, objective acid-base definition of asphyxia?

Richard B. Clark, MD

Departments of Anesthesiology and Obstetrics/Gynecology, University of Arkansas for Medical Sciences, 4301 West Markham, Mail Slot 515, Little Rock, AR 72205-7199

Reply

To the Editors: We appreciate Dr. Clark's interest in our article. His questions of how one defines "fetal asphyxia" and "what is the scientific, objective acid-base definition of asphyxia" are important ones. But they are not easily answered. There is no official consensus as to the definition of asphyxia. *Dorland's Illustrated Medical Dictionary,* twenty-sixth edition, 1985, indicates that the Greek derivation of asphyxia means "a stopping of the pulse." Asphyxia is defined as "a condition due to lack of oxygen in respired air, resulting in impending or actual cessation of apparent life." *Stedman's Medical Dictionary,* twenty-fifth edition, 1990, offers a less severe interpretation of asphyxia. This dictionary defines asphyxia as "impaired or absent exchange of oxygen and carbon dioxide on a ventilatory basis; combined hypercapnia and hypoxia or anoxia." These contemporary medical dictionaries provide two very different definitions. Considering the ambiguities of the term "asphyxia," it's not surprising that there are no universally accepted pH, Po_2, or base deficit cutoff values defining its presence. Such cutoffs by necessity would be arbitrary, similar to defining anemia on the basis of packed red blood cell volume. One solution is the utilization of a degree of acidosis and hypoxia represented by 2 SDs below the mean or below the fifth percentile. (On the basis of elective cesarean sections at our institution the mean −2 SDs for umbilical artery values are: pH 7.21, base deficit −4 mEq/L, and oxygen tension 8.0 mm Hg). Another possible definition, as suggested by Dr. Clark, would be a buffer base of <34 mmol/L, cited by Low as the degree of acidosis associated with an increased risk of neurologic abnormality.[1] This would be our preference for present-day usage.

But there are very few other studies available concerning the clinical significance of birth acidosis and hypoxia. Ideal end-points for defining "severe acidosis" or "severe hypoxia" will be those that help to distinguish neonates who have a high risk of long-term complications from those with a favorable outlook. As cord blood acid-base analysis becomes more widely used, we are confident that clinically useful end points will be established.

John W. C. Johnson, MD, and Douglas S. Richards, MD

University of Florida College of Medicine, Department of Obstetrics and Gynecology, JHMHC, Box J-294, Gainesville, FL 32610-0294

REFERENCE

1. Low JA. The role of blood gas and acid-base assessment in the diagnosis of intrapartum fetal asphyxia. AM J OBSTET GYNECOL 1988;159:1235-40.

Cocaine effects on fetal behavioral state

To the Editors: We read with great interest the article by Hume et al. (Hume RF Jr., O'Donnell KJ, Stanger CL, Killam AP, Gingras JL. In utero cocaine exposure: Observations of fetal behavioral state may predict neonatal outcome. AM J OBSTET GYNECOL 1989;161:685-90). We agree in theory with their hypothesis that abnormal neonatal behavioral state organization may be caused by in utero cocaine effects and that the neonatal abnormalities may be predated, and possibly predicted, by fetal behavioral state abnormalities. We have difficulty, however, in how their conclusions are reached. They describe their "fetal neurobehavioral profile," a rather elegant and painstaking examination that permits assessment and recording of the components of fetal behavior (fetal heart rate variability, breathing, gross body movement, and eye, chin, and mouth movement). Their assessment includes undisturbed organization and regulation as well as fetal self-regulation after acoustic stimulation. They define organization and regulation as "the coordination of related components of fetal behavior in a stable yet responsive state" and describe two such states, active sleep (rapid eye movements, chin motion, breathing, and reactive heart pattern) and quiet sleep (no eye movements, chin motion, or breathing with a nonreactive heart rate pattern). These states appear similar to, if not synonymous with, fetal states 2F and 1F, respectively, as described by Nijhuis et al.[1] The investigators then report fetal state disorganization, defined in the discussion as a lack of coincidence of the behavioral state components or delay in their integration, in 13 of the 20 fetuses serially evaluated in utero.

We have several questions regarding their definition of disorganization. Does it require total dissociation of the components of either or both of the fetal states

described during the entire examination, failure to maintain the stable association for a minimal portion of the examination, or failure to reorganize after the acoustic stimulation? Did meeting this definition during one of the serial evaluations place the fetus in the "disorganized fetal state" category, or did this require disorganization during all the examinations? How many examinations were performed, and over what range of gestation? How long were the examinations? By what gestation was regulation considered delayed?

In addition, there is some question as to what is normal, since a normal drug-free group was not evaluated with the neurobehavioral profile. In Nijhuis et al.'s original work,[1] before 38 weeks, coincidence, or simultaneous occurrence, of 1F and 2F state parameters occurred only 55% to 65% of the entire observation time, with the observation period lasting 112 minutes on the average. They suggested that these periods of coincidence occurred by chance and did not represent organized behavioral states because of the lack of simultaneous change of the state variables. Only with the synchronization of these changes, which developed around 36 to 38 weeks, could true behavioral states be defined. One can see how a preterm fetus, without periods of coincidence of parameters a considerable portion of the time, might be at risk for being classified as "disorganized." This is especially true if the fetus is delivered preterm, as were 25% of the fetuses in the study of Hume et al.

Further clarification of the definitions of "organized" and "disorganized" fetal states, as well as comparison with the fetal neurobehavioral profiles and neonatal outcomes of drug-free controls, would greatly enhance this investigation of the effects of cocaine on the developing central nervous system.

Bannie L. Tabor, MD, and Alex Soffici, MD
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Harbor-UCLA Medical Center, 1000 W. Carson St., Torrance, CA 90509

REFERENCE

1. Nijhuis JG, Prechtl HFR, Martin CB, Bots RSGM. Are there behavioral states in the human fetus? Early Hum Dev 1982;6:177-95.

Reply

To the Editors: The questions raised by Drs. Tabor and Soffici are insightful criticisms of our work. The issues addressed point out the potential for confusion concerning the terminology used to define the abnormal fetal behavior observed in our cocaine-exposed subjects. The pioneering work by Prechtl, Nijhuis, and Arduini and their colleagues (references 8, 9, 12, 14, and 15 of our article) were used in the formulation of our tool. The classification of fetal behavioral states follows that of Nijhuis, as stated. Our study design involved serial ultrasonographic observations (four to eight per subject) of at least 30 minutes' duration during the course of prenatal care. We scored the behavior observed from 28 weeks on to term. The fetal behavior observed included the acquisition and coordination of components of behavior into the defined states, as described by Nijhuis, and the ability and character of fetal reaction to acoustic stimulus, as well as atypical behavior not observed in normal subjects or described by others. Delay of state organization was scored as abnormal if stable state regulation failed to be appreciated by term (>38 weeks).

The confusion of terminology is significant, and Drs. Tabor and Soffici are entitled to a clarification of our use of "organized" and "disorganized." Normally organized and regulated fetal behavior follows the synchronization of components into stable states by 36 to 38 weeks of gestation. The "disorganized" classification applied to fetuses who had delay of organization, bizarre or atypical behavior, or disrupted behavior in response to stimulation. Three fetuses exhibited sustained hyperpnea plus lack of stable state organization, three fetuses exhibited recurrent yawning and abnormal response to stimulation, and three fetuses were hypertonic and hyperirritable, while four were never observed to attain organized state behavior by term gestation. The atypical fetal behaviors were present in those infants scored as being more severely affected as neonates.

The fetal response to stimuli mimics the "all-or-none" or "wired" neonate born with active cocaine intoxication. These neonates are very difficult to arouse, but once so they are very difficult to console. Bradycardia, ballistic fetal activity, and lack of habituation in response to acoustic stimulation were the fetal antecedents to such abnormal neonatal neurobehavior. We have shown our video recordings to many observers of various levels of ultrasonographic or neurobehavior sophistication and all agree in scoring the abnormal cases—the behavior really looks unusual. The need for a prospective study with normal control subjects is apparent and is under way. The use of video recording to score the fetal behavior affords a blinded evaluation to address the criticism of the subjective nature of this interpretation. The results of a larger-prospective longitudinal cohort study are needed to validate the fetal neurobehavioral profile's usefulness and reliability in a clinical setting.

I appreciate the interest in our work.

Roderick F. Hume, Jr., MD, MAJ, MC, USA
Department of Obstetrics and Gynecology, Walter Reed Army Medical Center, Washington, DC 20307-5001

Preeclampsia—An endothelial cell disorder plus "something else"?

To the Editors: We enjoyed the article by Roberts et al. (Roberts JM, Taylor RN, Musci TJ, et al. Preeclampsia: An endothelial cell disorder. AM J OBSTET GYNECOL 1989;161:1200-4). This work brings up to date the ideas of Page[1] (he also worked in San Francisco) and Beker.[2] Their concept was one of uteroplacental ischemia as the underlying pathophysiologic characteristic of preeclampsia. We agree with the idea that the clinical

syndrome of preeclampsia arises when placental perfusion is poor. In some instances there may be underlying vascular or autoimmune disease.[3, 4] We suggest that in many primigravid women the poor placental perfusion resulting from a failure of secondary trophoblast invasion seems to be related to a genetic,[5, 6] or possibly immunogenetic,[7] susceptibility. Roberts et al. do not offer any explanation either for the fact that preeclampsia is commonest in primigravid patients or for the protective effect of a first pregnancy.[8] Does this not suggest an involvement of the immune system,[9] and if so, how?

W. A. Liston

Simpson Memorial Maternity Pavilion, Lauriston Place, Edinburgh, Scotland EH3 9EF

D. C. Kilpatrick, BSc, PhD

Blood Transfusion Department, Royal Infirmary of Edinburgh, Edinburgh, Scotland EH3 9EF

REFERENCES

1. Page EW. The relationship between hydatid moles, relative ischemia of the gravid uterus, and the placental origin of eclampsia. AM J OBSTET GYNECOL 1939;37:291-3.
2. Beker JC. Aetiology of eclampsia. J Obstet Gynaecol Br Emp 1948;55:756-65.
3. McCartney CP. Pathological anatomy of acute hypertension of pregnancy. Circulation 1964(suppl);2:37-42.
4. Kilpatrick DC, McLean C, Liston WA, Johnstone FD. Antiphospholipid antibody syndrome and pre-eclampsia. Lancet 1989;2:987-8.
5. Cooper DW, Liston WA. Genetic control of severe pre-eclampsia. J Med Genet 1979;16:409-16.
6. Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: possible single gene control of severe pre-eclampsia in the descendants of eclamptic women. Br J Obstet Gynaecol 1986;95:644-53.
7. Kilpatrick DC, Liston WA, Gibson F, Livingstone J. Association between susceptibility to pre-eclampsia within families and HLA DR.4. Lancet 1989;2:1063-4.
8. McGillivray I. Some observations on the incidence of pre-eclampsia. J Obstet Gynaecol Br Emp 1958;65:536-9.
9. Redman CWG. Immunology of the placenta. Clin Obstet Gynecol 1986;13:469-99.

Reply

To the Editors: Drs. Liston and Kilpatrick correctly point out that our hypothesis extends the role of reduced placental perfusion in the development of the multisystemic syndrome of preeclampsia, a concept originally proposed by the late Professor E. W. Page of this department. Our proposal provides a rationale for the communication of this abnormality in the intervillous space to the many organ systems involved in the syndrome. As pointed out, we feel that endothelial cell injury-activation by an agent(s) produced by poorly perfused trophoblast could explain the systemic abnormalities observed in preeclampsia. Our hypothesis does not differentiate the several causes of reduced perfusion but predicts that they converge to a common pathway, resulting in the production of this bioactive agent(s).

It was not our purpose to explain how this reduced perfusion is initiated in first-pregnancy preeclampsia. We, as others, unfortunately have no direct information to identify the inciting pathogenetic event in this enigmatic disease. As Drs. Liston and Kilpatrick have demonstrated, there is good evidence that the "something else" described in their letter involves a combination of genetic and immunologic factors.

We feel strongly that any candidate as the intiating cause(s) of preeclampsia must account for reduced trophoblastic invasion, genetic predisposition to the disorder, and the protective effect of exposure to paternal antigens, usually by a previous pregnancy. These phenomena may derive sequentially from the same primary process. For example, the immunogenetic dissimilarity of mother and father, or mitigation of maternal immune "aggression" by previous exposure to paternal antigens, could influence the production of factors (e.g., blocking antibodies, activators of suppressor cells, etc.) that immunologically protect the trophoblast. If these factors are not present in a requisite amount, trophoblastic invasion may be retarded as part of an allogeneic rejection phenomenon. Alternatively, it is possible that independent mechanisms interact to produce preeclampsia. In this scenario a genetically determined abnormality of trophoblast function reducing placental invasiveness might lead to preeclampsia only in the first pregnancy because it compounds a subtle form of the immunologic compromise proposed above. There are obviously numerous other combinations of independent factors (including preexistent microvascular disease) that could converge to generate the syndrome.

Recent advances in our understanding of the fetal-maternal interface, such as the recognition of unique placental major histocompatibility antigens[1] and the characterization of enzymes involved in trophoblastic invasion,[2] should accelerate our understanding of the complexities of normal and abnormal placentation and ultimately will allow the precise identification of "something else."

James M. Roberts, MD, and Robert N. Taylor, MD, PhD

Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, Room M-1489, San Francisco, CA 94143

REFERENCES

1. Kovats S, Main EK, Librach C, Stubblebine M, Fisher S, DeMars R. A class I antigen, HLA-G, expressed in human trophoblasts. Science 1990;248:220-3.
2. Fisher SJ, Cui T-Y, Zhang L, et al. Adhesive and degradative properties of human placental cytotrophoblast cells in vitro. J Cell Biol 1989;109:891-902.

Possible therapeutic applications of cordocentesis

To the Editors: We read with interest the journal articles by Drs. Economides and Nicolaides et al. concerning antenatal detection of different metabolic disturbances in small-for-gestational-age (SGA) fetuses in samples obtained by cordocentesis.[1-4] The data provided by these authors suggest reduced glucose supply as a reason for reduced growth,[1] and show lower fetal insulin/glucose ratio,[2] and elevated fetal glycine/valine ratio,[3] in SGA fetuses. Most recently they showed that fetal hypertriglyceridemia may be used as a marker for hypoxemia in SGA fetuses.[4]

Intrauterine growth retardation is second only to prematurity in its association with perinatal morbidity and mortality. It may well be time to rekindle interest

in potential clinical application of cordocentesis—namely hyperalimentation as alluded to briefly by Weiner.[5] Because of its invasiveness and concern for infection and possible bleeding, this procedure may be of importance in the management of only a limited group of very premature SGA fetuses caused by uteroplacental insufficiency. Inasmuch as these fetuses often have reduced fetal movements, the ease of this procedure might be enhanced.

Yet to be defined, probably with animal models, are the frequency and specific nutrients that would benefit these fetuses. Thought might be given to a chronic indwelling silastic catheter for continuous nutrition access similar to the Hickman catheter widely used for total parenteral nutrition.

Another therapeutic potential of cordocentesis could be direct administration of steroids to enhance fetal pulmonary maturation. This would bypass the placenta, thus possibly shortening the 12 to 14 hour period that current maternal intramuscular treatment requires before beneficial changes are noted. We would be very interested to learn the above authors' views on these potential clinical therapeutic applications that may widen the current therapeutic uses of cordocentesis.

David M. Sherer, MD, and Jacques S. Abramowicz, MD

Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Box 668, Strong Memorial Hospital, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Rochester, NY 14642

REFERENCES

1. Economides DL, Nicolaides KH. Blood glucose and oxygen tension in small-for-gestational-age fetuses. AM J OBSTET GYNECOL 1989;160:385-9.
2. Economides DL, Proudler A, Nicolaides KH. Plasma insulin in small-for-gestational-age fetuses. AM J OBSTET GYNECOL 1989;160:1091-4.
3. Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Bottoms S, Evans M. Cordocentesis in the diagnosis of intrauterine starvation. AM J OBSTET GYNECOL 1989;161:1004-8.
4. Economides DL, Crook D, Nicolaides KH. Hypertriglyceridemia and hypoxemia in small-for-gestational-age fetuses. AM J OBSTET GYNECOL 1990;162:382-6.
5. Weiner CP. The role of cordocentesis in fetal diagnosis. Clin Obstet Gynecol 1988;285-95.

Response declined

Serum levels of interleukin-2 and tumor necrosis factor-α in women with recurrent abortion

To the Editors: From an immunologic point of view a certain part of recurrent abortions must be considered as a host-versus-graft reaction of the mother against the fetus. Inasmuch as elevated interleukin-2 levels were found during pregnancy[1] and elevated tumor necrosis factor-α levels were found during acute rejection crisis of allotransplants,[2] we determined levels of interleukin-2 and tumor necrosis factor-α in women with recurrent abortions. During weeks 6 and 14 of pregnancy in 92 women with at least three abortions, 42 pregnant women without any complications, and 28 healthy nonpregnant controls, serum samples were taken and analyzed by a radioimmunoassay (IRE Medgenix, Belgium), allowing the detection of 0.1 ng/ml tumor necrosis factor-α and 0.5 unit interleukin-2 per milliliter. Statistical analysis was performed with the Student t test.

We found an increase in tumor necrosis factor-α serum levels ($p < 0.05$) and a decrease in interleukin-2 serum levels ($p < 0.05$) in women with recurrent abortion compared with women with normal pregnancies (Table I). In 52% of women with recurrent abortion but in only 24% of normal pregnant women no interleukin-2 serum levels could be detected. As far as it could be considered by our study, no differences concerning age or number of pregnancies could be observed.

In accordance with these results one can presume that disturbances of maternal immune tolerance of the fetus in the meaning of rejection could be observed by increased tumor necrosis factor-α serum levels. Low serum levels of interleukin-2 in women with recurrent abortion could be associated with a lack of T and B cell growth, promoting effects that probably lead to insufficient induction of a pregnancy, protecting maternal immune response.

Peter Mallmann, Andreas Werner, and Dieter Krebs

Department of Gynecology and Obstetrics, University of Bonn, Bonn, West Germany 53000

REFERENCES

1. Favier R, Edelman P, Mary JY, Sadoul G, Douay L. Presence of elevated serum interleukin-2 levels in pregnant women. N Engl J Med 1990;322:270.
2. Hammer C. Preliminary studies of lymphokines in heart transplanted patients. In: Clinical usefulness of cytokines radio-immuno assays. Brussels: IRE, 1988.

What is birth asphyxia?

To the Editors: We read the article by Thorp et al. (Thorp JA, Sampson JE, Parisi VM, Creasy RK. Routine umbilical cord blood gas determinations? AM J OBSTET GYNECOL 1989;161:600-5.) with interest. This has long been a concern of ours.[1] We believe intensive investigation of cord-blood acid-base findings will substan-

Table I. Tumor necrosis factor-α and interleukin-2 serum levels in women with recurrent abortions

Group	*No.*	*Interleukin-2 (U/ml) (mean ± SD)*	*Tumor necrosis factor-α (ng/ml) (mean ± SD)*	*p Value*
Control	28	0.88 ± 6.2	0.98 ± 7.2	—
Normal pregnancy	42	5.3 ± 3.8	0.94 ± 5.6	0.05
Recurrent abortion	92	0.5 ± 0.74	15.8 ± 14.2	0.05

tially assist in unraveling the mysteries of neonatal physiology and will also assist in the legal arena by elucidating cause and effect of subsequent infant neurologic outcome and by clarifying the result of actions by the medical practitioner.

We were somewhat disturbed by the use of the term *birth asphyxia*. What is birth asphyxia? The authors did not define it, yet make statements such as ". . .acidemia is the most sensitive indicator of birth asphyxia at the time of delivery" and ". . .umbilical cord pH is also useful in ruling out the diagnosis of birth asphyxia in the depressed newborn." Can it be diagnosed only with a pH? How can one rule out a diagnosis if it is not defined?

Asphyxia literally means "a stopping of the pulse."[2] In common usage it implies suffocation, a lack of oxygen, and probably an increase in carbon dioxide tension. That definition was sufficient until blood gas monitoring became available, but is it good enough now? Can asphyxia be present without hypercarbia? Is an anoxic infant necessarily acidemic? The definition of asphyxia that we prefer is "acidosis, hypoxia and hypercarbia."[3] Granted, there are rare instances in which hypoxia could be present without hypercarbia. Actually, all infants are asphyxiated at birth (acidotic, hypoxic, hypercarbic)! The question is what degree of physiologic derangement must be present to cause depression (Apgar score <7)? The authors defined a normal umbilical artery pH as the mean minus 2 SD (the lower limit of normal would then be 7.10). A pH of 7.10 would seem to be a very low finding indeed, and yet be considered normal.

The fact that a majority of the depressed infants had a normal pH (77.8%) and 2.1% of the vigorous infants exhibited umbilical artery acidemia supports the notion that the Apgar score should be used as it was intended—to identify the neonate that needs resuscitation; it should and must never be used as an objective evidence of asphyxia. Again, the finding that the majority of the depressed infants had a normal pH makes one wonder about the sensitivity of pH as a test.

Birth asphyxia is a term that we believe should be discarded. Thorp et al. probably did not define it for good reason—it is probably impossible and undesirable to define in these days of greater precision. In the past, whenever an infant was born in a depressed state, the child was said to be experiencing birth asphyxia. No more. Drs. Silverman[4] and Yeomans[5] do not even use the term *birth asphyxia* (except in their references), and Dr. Page[6] used it sparingly.

We urge Drs. Thorp et al. to either define the term (can pH alone be used?) or abandon it (except in a historical sense).

Richard B. Clark, MD, and J. Gerald Quirk, MD, PhD
Departments of Anesthesiology and Obstetrics/Gynecology, University of Arkansas, 4301 W. Markham, Mail Slot 515, Little Rock, AK 72205-7199

REFERENCES

1. Clark RB, Cooper JO, Stephens SR, Brown WE. Neonatal acid-base studies. I. The effect of normal labor and obstetrical manipulation. Obstet Gynecol 1969;33:23.
2. Taylor EJ, ed. Dorland's illustrated medical dictionary. Philadelphia: WB Saunders, 1988.
3. Albright GA, et al. Birth asphyxia and neonatal resuscitation. In: Anesthesia in obstetrics. Boston: Butterworth, 1986:567.
4. Silverman F, et al. The Apgar score: is it good enough? Obstet Gynecol 1985;66:331-6.
5. Yeomans ER, et al. Umbilical cord pH, P_{CO_2}, and bicarbonate following uncomplicated term vaginal deliveries. AM J OBSTET GYNECOL 1985;151:798.
6. Page FO, et al. Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings. AM J OBSTET GYNECOL 1986;154:1306.

Reply

To the Editors: We appreciate the authors' interest in our research. It seems rather naive to hope that the term *birth asphyxia* will disappear. Birth asphyxia is variously defined by numerous authors as you point out. It may be defined as acidemia, hypercarbia, and hypoxia.[1] On the other extreme, birth asphyxia may be more rigidly defined to include biochemical criteria (hypoxia, hypercarbia, acidemia) and clinical criteria (birth depression and end-organ evidence of hypoxic damage in the newborn period e.g., early neonatal seizure, renal or cardiac dysfunction).[2] Birth asphyxia, by anyone's definition, at the very least must include acidemia. A pH ≤2 SD below the mean for a population is a satisfactory definition for acidemia. Then, an umbilical cord arterial Ph of ≤7.10 is a reasonable cut-off according to the larger studies[3,4] and certainly is consistent with the observation that the typical neonatal sequelae of birth asphyxia rarely occur at a pH above this value.[5]

The following statement: "the finding that the majority of the depressed infants had a normal pH makes one wonder about the sensitivity of the pH as a test," suggests that the authors have missed a very important point. If the pH is testing for neonatal depression then certainly it is insensitive. However, the pH is not testing for neonatal depression. The pH is testing for birth asphyxia, and it is extremely sensitive because it has no false-negative results (i.e., birth asphyxia does not occur in the presence of a normal pH). Umbilical cord blood pH is not specific because it has a very high false-positive rate (i.e., the majority of babies born with cord blood acidemia develop normally).[6] The finding that the majority of depressed infants had a normal pH should not make one wonder about the sensitivity of pH as a test; rather, it underscores the important fact that there are many causes of neonatal depression other than birth asphyxia (e.g., drugs, trauma, aspiration of pharyngeal secretions, neonatal manipulation, congenital anomalies, antepartum fetal insults, and prematurity).

James A. Thorp, MD
St. Lukes Hospital, 4400 Wornall Road, Kansas City, MO 64111

REFERENCES

1. Creasy RK, Resnik R, eds. Maternal-fetal medicine: principles and practice. Philadelphia: WB Saunders, 2nd ed, 1988:318.
2. Hill A, Volpe JJ. Perinatal asphyxia: clinical aspects. Clin Perinatol 1989;16:435-57.
3. Thorp JA, Sampson JE, Parisi VM, Creasy RK. Routine

umbilical cord blood gas determinations? AM J OBSTET GYNECOL 1989;161:600-5.
4. Sykes GS, Johnson P, Ashworth F, et al. Do Apgar scores indicate asphyxia? Lancet 1982;1:494-6.
5. Assessment of fetal and newborn acid-base status. ACOG Tech Bull April 1989, no 127.
6. Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. Br Med J 1988;297:24-7.

Embryo migration responsible for ectopic pregnancies

To the Editors: We read with interest the case reported by Weyerman and colleagues (Weyerman PC, Verhoeven AThM, Alberda ATh. Cervical pregnancy after in vitro fertilization and embryo transfer. AM J OBSTET GYNECOL 1989;161:1145-6.). The authors suggest that manipulation of the cervical canal, as in embryo transfer, is important in the etiology of cervical pregnancy after in vitro fertilization. We believe that embryo migration, rather than cervical manipulation, is responsible for the different forms of ectopic and heterotopic pregnancies in patients at high risk for ectopic implantation.

We have reported a case of cervical pregnancy after gamete intrafallopian transfer,[1] which involves no cervical manipulation. With regard to management of the cervical pregnancy, the confirmation of the diagnosis was made late in the pregnancy, thereby making its termination more difficult and hazardous. Therefore the authors allowed the pregnancy to continue, a situation that finally caused hysterectomy to become necessary with loss of reproductive function.

We believe that ultrasonography, particularly transvaginal, will enable early diagnosis of cervical pregnancy after in vitro fertilization or gamete intrafallopian transfer. Once the diagnosis is confirmed, termination of the pregnancy surgically, as in our case,[1] or with methotrexate[2] should be offered to the patient because the risk of hemorrhage is much higher after 8 weeks' gestation.

Botros Rizk, MB, BCh, and Peter Brinsden, MB, BS
The Hallam Medical Centre, 112 Harley St., London, England WIN IAF

REFERENCES

1. Brinsden PR, Smith B, Craft IL. Cervical ectopic pregnancy following gamete intrafallopian transfer. J In Vitro Fertil Embryo Transfer 1988;5:370-2.
2. Palti Z, Rosenn B, Goshen R, Ben-Chitrit A, Yagel S. Successful treatment of a viable cervical pregnancy with methotrexate. AM J OBSTET GYNECOL 1989;161:1147-8.

Response declined

Responsibility expands to extended family members

To the Editors: Neonatal alloimmune thrombocytopenia, the platelet equivalent of hemolytic disease of the newborn, is a result of maternal antiplatelet antibodies formed against incompatible fetal platelet antigens.[1-5] At least 80% of cases are a result of a lack of the PL-A1 antigen on maternal platelets, which is present on the paternal and affected infant platelets.[2, 6] Although much about this entity is not well understood, it occurs in 1 of 2000 to 1 of 5000 live births, with high rates of morbidity and mortality from the intracranial hemorrhage that occurs in 10% to 40% of the affected infants.[1-7] Because 25% to 50% of the often severe intracranial hemorrhages may occur antenatally,[3-4] current efforts have been expanded to include in utero treatment as well as postnatal intervention.[1, 3, 4, 6] The extremely high risk (>85%) of subsequent pregnancies also being affected underlines the importance of correct diagnosis of the infant, with appropriate family counseling and education.[1, 2]

An inquiry from the mother of one of our recent neonatal intensive care unit graduates diagnosed with neonatal alloimmune thrombocytopenia has highlighted our responsibility to obtain a complete family pedigree and offer genetic counseling and appropriate platelet screening to extended family members of such infants. In this case, the pregnant maternal aunt of the affected infant requested information regarding the chances of her unborn child being affected with neonatal alloimmune thrombocytopenia. Information regarding the disease manifestations and risks to future pregnancies had been discussed with the parents of the index case at the time of diagnosis. However, formalized genetic counseling had not been offered to extended family members.

Some sources recommend obtaining a family pedigree to identify possible PL-A1 antigen-negative platelet donors for future affected infants.[2] The few references that recommend family pedigree and complete platelet testing of all female relatives of the affected infant's mother to identify more women at risk of delivery of an infant with neonatal alloimmune thrombocytopenia are found mainly in the hematologic literature.[6] Because this literature is not generally read by obstetricians, perinatologists, and neonatologists, it was deemed pertinent to alert these medical care providers to the need for family pedigree-targeted platelet antigen testing and genetic counseling in families of infants with neonatal alloimmune thrombocytopenia.

Current research on neonatal alloimmune thrombocytopenia—its cause, clinical course, and antenatal and postnatal treatment—indicates that both antenatal and immediate postnatal treatment may considerably lessen the morbidity and mortality rates of this disorder.[1-4, 6, 7-9] For this reason, we who have direct contact with families of such infants should make extra efforts to identify all females at risk of the delivery of an affected infant.

Kay L. Saving, MD, Rodney A. McLaren, MD, Tim C. Miller, MD, and Mary O'Connor, MSSW
Departments of Pediatrics and Obstetrics and Gynecology, University of Illinois, One Illini Dr., Box 1649, Peoria, IL 61656

REFERENCES

1. Kaplan C, Daffos F, Forestier F, et al. Management of alloimmune thrombocytopenia: antenatal diagnosis and in utero transfusion of maternal platelets. Blood 1988; 72:340-3.
2. Blachette VS. Neonatal alloimmune thrombocytopenia. In:

Stockman JA III, Pochedly C, eds. Developmental and neonatal hematology. New York: Raven Press, 1988:145.
3. Lynch L, Bussel J, Goldberg JD, et al. The in utero diagnosis and management of alloimmune thrombocytopenia. Prenat Diagn 1988;8:329-31.
4. Bussel JB, Berkowitz RL, McFarland JG, Lynch L, Chitkara U. Antenatal treatment of neonatal alloimmune thrombocytopenia. N Engl J Med 1988;319:1374-8.
5. Beck R, Reid DM, Lazarte R. Intravenous gammaglobulin therapy for neonatal alloimmune thrombocytopenia. Am J Perinatol 1988;5:79-82.
6. Reznikoff-Etievant MF. Management of alloimmune neonatal and antenatal thrombocytopenia. Vox Sang 1988; 55:193-201.
7. Morales WJ, Stroup M. Intracranial hemorrhage in utero due to isoimmune neonatal thrombocytopenia. Obstet Gynecol 1985;65:20S.
8. Friedman JM, Aster RH. Neonatal alloimmune thrombocytopenic purpura and congenital porencephaly in two siblings associated with a "new" maternal antiplatelet antibody. Blood 1985;65:1412-5.
9. Bussel J, Berkowitz, McFarland J, Lynch L, Chitkara U. In-utero platelet transfusion for alloimmune thrombocytopenia. Lancet 1988;1:1307-8.

Does prophylactic amnioinfusion make a difference?

To the Editors: I read the recent article by Strong et al. (Strong TH, Hetzler G, Paul RH. Amniotic fluid volume increase after amnioinfusion of a fixed volume. Am J Obstet Gynecol 1990;162:746-8) with great interest.

I do have some questions about the article. First, why did they feel the need to warm the normal saline solution to 37° C? Miyazaki and Taylor[1] noted that the blood warmer "proved to be unnecessary as long as the infusion rate was limited to 10 to 15 ml per minute."

Second, the authors state, "the mean change in amniotic fluid index for those with preinfusion amniotic fluid index values of >5.0 cm (n = 15) was 4.0 ± 0.8 cm." Why would they amnioinfuse patients who do not meet the criteria of oligohydramnios[2]? Moreover, both Rutherford et al.[3] and Sarno et al.[4] showed adverse intrapartum outcome among patients with amniotic fluid index values ≤5.0 cm, not ≥5.0 cm.

Third, and most important, does prophylactic saline solution amnioinfusion among patients with amniotic fluid index values ≤5.0 cm decrease the incidence of recurrent severe variable fetal heart rate decelerations and the associated morbidity of cesarean section for fetal distress?

Suneet P. Chauhan, MD
USNH Naples, Italy, Box 19, FPO New York, NY 09521-0700

REFERENCES

1. Miyazaki FS, Taylor NA. Saline amnioinfusion for relief of variable or prolonged decelerations. Am J Obstet Gynecol 1983;146:670-8.
2. Phelan JP, Smith CV, Broussard P, et al. Amniotic fluid volume assessment with the four-quadrant technique at 36-42 weeks gestation. J Reprod Med 1987;32:540-2.
3. Rutherford SE, Phelan JP, Smith CV, et al. The four-quadrant assessment of amniotic fluid volume: ad adjunct to antepartum fetal heart rate testing. Obstet Gynecol 1987;70:353-6.
4. Sarno AP, Ahn MO, Harbinder SB, et al. Intrapartum Doppler volecimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. Am J Obstet Gynecol 1989;1508-14.

Reply

To the Editors: We appreciate the attention and comments directed to our manuscript by Dr. Chauhan.

During our study the infusion rate approached 20 ml/min, thus exceeding Miyazaki and Taylor's maximum recommended rate for infusion of room temperature saline solution. When the amnioinfusate bottle is held 3 to 4 feet above the infusion catheter tip, as it is during most amnioinfusion procedures, the rate of infusion will be 20 ml per minute.[1] Therefore it can be expected that a significant number of women who receive gravity-flow amnioinfusion will exceed the "15 ml rule" and thereby be at increased risk if room temperature saline solution issued. Whereas slower infusion rates can be used, the initial bolus of fluid should be infused relatively rapidly to be effective. If the amnioinfusate bag is held <3 feet above the catheter tip, the rate of amnioinfusion may be too slow to have clinical utility, especially in the setting of severe variable fetal heart rate decelerations or bradycardia. Moreover, reversal of flow through the infusion catheter can occur if the saline solution bag is held so low that intrauterine pressure exceeds that of the incoming saline solution.

To answer the second question, our study protocol for prophylactic amnioinfusion stipulated that each subject was to receive infusion until an amniotic fluid index of at least 8 cm was obtained. Rutherford and associates[2] showed that intraobserver and interobserver error in determining the amniotic fluid index can be as large as 2 cm. An amniotic fluid index ≥8 cm was chosen as a goal because it was above the 5 cm "at risk" level and well outside the range of measurement error. As amnioinfusion appears to be a relatively safe procedure, augmenting the amniotic fluid index to 8 cm likely does no harm and provides an extra margin of safety against intrapartum leakage. Despite this, an amniotic fluid index of 8 cm falls well below the mean for third-trimester gestations.[3, 4]

Does prophylactic amnioinfusion make a difference? Clearly. In a randomized clinical trial of 60 laboring women with amniotic fluid index values ≤5 cm, the amnioinfused group had significantly lower rates of meconium passage, severe variable decelerations, end-stage bradycardias, and operative intervention for fetal distress.[5] In addition, significantly higher umbilical arterial pH values were noted in the infusion group.

As Dr. Chauhan points out, Sarno and associates[6, 7] have suggested that the laboring gravida with an amniotic fluid index ≤5 cm is at significantly increased risks of adverse fetal outcome. Combination of early

intrapartum amniotic fluid index assessment with prophylactic amnioinfusion might favorably impact the outcome of oligohydramnic fetuses. Rather than using severe variable decelerations or bradycardia to trigger amnioinfusion, Sarno's "fetal admission test" may identify early in labor those in whom ominous patterns are likely to develop at a time when amnioinfusion can be done under more controlled, relaxed conditions. Therefore, we propose that detection and correction of oligohydramnios early in labor has more utility than a protocol that waits for the appearance of ominous patterns before reacting. With the knowledge that 250 ml of normal saline solution will increase the amniotic fluid index by roughly 4 cm, the practitioner can use amnioinfusion more effectively by titrating the infusion to an appropriate amniotic fluid index.

Thomas H. Strong, Jr., MD
Phoenix Perinatal Associates, 1300 N. 12th, #320, Phoenix, AZ 85006

Gretchen Hetzler, MD, and Richard H. Paul, MD
Department of Obstetrics and Gynecology, Los Angeles County/University of Southern California Women's Hospital, 1240 N. Mission Road, Room 5K40, Los Angeles, CA 90033

REFERENCES

1. Miyazaki FS. Relieving variable decelerations. Contemp Obstet Cynecol 1987;29:33.
2. Rutherford SE, Smith CV, Phelan JP, Kawakami K, Ahn MO. Four-quadrant assessment of amniotic fluid volume. J Reprod Med 1987;32:587.
3. Rutherford SE, Phelan JP, Smith CV, Jacobs N. The four-quadrant assessment of amniotic fluid: an adjunct to antepartum fetal heart rate testing. AM J OBSTET GYNECOL 1987;70:353.
4. Moore TR, Cayle JE. The amniotic fluid index in normal human pregnancy. AM J OBSTET GYNECOL 1990;162:1168.
5. Strong TH, Hetzler G, Sarno AP, Paul RH. Prophylactic intrapartum amnioinfusion: a randomized clinical trial. AM J OBSTET GYNECOL 1990;162:1370.
6. Sarno AP, Ahn MO, Phelan JP. Intrapartum amniotic fluid volume at term: association of ruptured membranes, oligohydramnios and increased fetal risk. J Reprod Med 1990;35:719.
7. Sarno AP, Ahn MO, Brar HS, et al. Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. AM J OBSTET GYNECOL 1989;161:1508.

Cordocentesis and immune thrombocytopenia—Continued

To the Editors: I thank Dr. Daffos for his response[1] to my letter of inquiry[2] requesting references to support his conclusion that fetal blood sampling should be used in the evaluation of pregnancies complicated by autoimmune thrombocytopenia. My specific request was stimulated by his statement that "The occurrence of fetal bleeding accidents during pregnancy is more frequent in this condition (i.e., autoimmune thrombocytopenia) than in cases of alloimmunization, so it is important to know the fetal platelet count as early as possible to attempt to treat the fetus via maternal therapy." In response to my query, Dr. Daffos offered seven references to support this statement. I have reviewed each and find them lacking.

The first reference was a commentary in the *British Journal of Obstetrics and Gynaecology* concerning both autoimmune thrombocytopenia and alloimmune thrombocytopenia.[4] No where in the Discussion section do the authors suggest or report evidence of *fetal* hemorrhage as a result of autoimmune thrombocytopenia. They do reference several publications (some of which Dr. Daffos included). None of these publications documents a single instance of a *fetal* hemorrhage from autoimmune thrombocytopenia. All the untoward complications of autoimmune thrombocytopenia occurred in the neonatal period.

The second reference also appeared in the *British Journal of Obstetrics and Gynaecology*.[5] It reviews 51 pregnancies complicated by autoimmune thrombocytopenia. It includes six neonates delivered vaginally whose platelet counts were <50,000. As summarized on page 681 of that article, all bleeding complications occurred in the neonatal period.

The third reference offered[6] was a review article in *Annals of Internal Medicine*. Dr. Kelton effectively argues the opposite of Dr. Daffos that the outcome of pregnancies complicated by autoimmune thrombocytopenia is not as bad in prospective studies as suggested by isolated case reports. He cites his unpublished study in which only 10 of 94 live-born infants had a hemostatic defect and all bleeding complications occurred in the *neonatal period.* In a prior report on autoimmune thrombocytopenia (AM J OBSTET GYNECOL 1982;144:449), Dr. Kelton reported on 41 pregnancies in which there were five stillbirths or late abortions. Two women had lupus, two had preeclampsia, and the fifth had negative test results for platelet associated immunoglobulin G.

The fourth reference was cited erroneously.[7] The correct page number follows this letter. Drs. Hind et al. report a single case of autoimmune thrombocytopenia treated at 36 weeks' gestation with gammaglobulin. There were no fetal or neonatal bleeding complications. The authors then reviewed 18 reports of 159 deliveries published between 1973 and 1982. There were no instances of antenatal hemorrhage. All perinatal bleeding complications occurred in the neonatal period. Perinatal mortality was not broken into the fetal and neonatal periods. However, the perinatal loss rate and neonatal intracranial hemorrhage rate happen to be the same (2.4%).

The fifth reference[8] appeared in the *British Medical Journal.* This is a case report of a single fetus; the mother was treated with immune globulin before delivery. The neonatal platelet count was normal after spontaneous vaginal delivery.

The sixth reference[9] appeared in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY. These authors studied alloimmune thrombocytopenia and therefore it is not relevant for the assignment of risk from autoimmune thrombocytopenia.

The final reference that Dr. Daffos supplied appeared in *Obstetrics and Gynecology*.[10] This report reviews five cases. In case one the bleeding complications were limited to the neonatal period. In case two the history is more consistent with alloimmune thrombocytopenia inasmuch as maternal thrombocytopenia could never be documented. In case three the woman had a severe preeclampsia associated with thrombocytopenia and the fetus had a normal platelet count. In case four there was an intrauterine fetal death at 28 weeks' gestation when the patient's platelet count was normal. No evidence of fetal hemorrhage during the intrapartum period was apparently found. In case five there were no bleeding complications.

I do not suggest that it poses absolutely no risk to the fetus. I accept antenatal hemorrhage has probably occurred. However, it is hard to believe that if autoimmune thrombocytopenia posed a sizable *fetal* risk, antenatal hemorrhage would not already have been documented in the literature considering its frequency of occurrence. A rare loss should be expected because a sizable percentage of women with autoimmune thrombocytopenia have other disorders such as lupus. This disorder must be separated from alloimmune thrombocytopenia where the fetal risk is sizable.

In summary, none of the references provided by Dr. Daffos offers any support for his statement that autoimmune thrombocytopenia poses a significant risk to the fetus. To recommend cordocentesis without documentation as to what the risk of disease to the fetus is perpetuates a modern myth. Cordocentesis poses a small but finite fetal risk even in the best hands and complications of procedures performed for this indication have been reported.[11] We will all agree that during the neonatal period, autoimmune thrombocytopenia does pose a risk. However, most of the neonatal complications can be prevented by modern practices. There is no justification to place the fetus at greater risk by performing an unindicated, invasive procedure.

Carl P. Weiner, MD

Maternal Fetal Medicine, Department of Obstetrics and Gynecology, University of Iowa, Iowa City, IA 52242

REFERENCES

1. Daffos F. Am J Obstet Gynecol 1990;162:298.
2. Weiner CP. Thrombocytopenia and cordocentesis clarifications requested. Am J Obstet Gynecol 1989;161: 1091-2.
3. Daffos F, Forestier F, Kaplan C, Cox W. Prenatal diagnosis and management of bleeding disorders with fetal blood sampling. Am J Obstet Gynecol 1988;158:939-46.
4. Hegde UM. Immune thrombocytopenia in pregnancy and the newborn. Br J Obstet Gynaecol 1985;92:657-9.
5. Jones RW, Asher MI, Rutherford CJ, Munro HM. Autoimmune (idiopathic) thrombocytopenia purpura in pregnancy and the newborn. Br J Obstet Gynaecol 1977;84:679-83.
6. Kelton JG. Management of the pregnant patient with idiopathic thrombocytopenia purpura. Ann Int Med 1983; 99:796-800.
7. Hind C, Morfr P, Schneider H, Morell B. Idiopathische thrombozytopenische purpura in der schwangerschaft. Schweiz Med Wochenschr 1982;112:1389-95.
8. Morgenstern GR, Measday B. Hegde UM. Autoimmune thrombocytopenia in pregnancy: new approach to management. Br Med J 1983;287:584.
9. Sitarz AL, Driscoll JM, Wolff JA. Management of isoimmune neonatal thrombocytopenia. Am J Obstet Gynecol 1976;124:39-42.
10. Territo M, Finklestein J, Oh W, Hobel C, Kattlove H. Management of autoimmune thrombocytopenia in pregnancy and in the neonate. Obstet Gynecol 1973;41:579-84.
11. Moise KJ, Carpenter RJ, Cotton DB, Wasserstrum N, Kirshon B, Cano L. Percutaneous umbilical cord blood sampling in the evaluation of fetal platelet counts in pregnant patients with autoimmune thrombocytopenia purpura. Obstet Gynecol 1988;72:346-50.

Response declined

Maternal serum unconjugated estriol levels are lower in the presence of fetal Down syndrome

To the Editors: The recent article by Macri and his colleagues (Macri JN, Kasturi RV, Krantz DA, Cook EJ, Sunderji SG, Larsen JW. Maternal serum Down syndrome screening: unconjugated estriol is not useful. Am J Obstet Gynecol 1990;162:672-3) has prompted us to summarize all the published data sets that have come to our attention that examine the relation between unconjugated maternal serum estriol levels in the second trimester and fetal Down syndrome (affected pregnancies). Table I lists the study site, number of affected pregnancies, and median unconjugated estriol multiples of the median in affected pregnancies. An additional data set that includes 54 affected pregnancies with a median unconjugated estriol of 0.71 multiples of the median has come to our attention (MacDonald M, Wagner R. Genetrix, Scottsdale, Ariz.

Table I. Median maternal serum unconjugated estriol levels in pregnancies with fetal Down syndrome in the second trimester

Site and reference	*No. of affected pregnancies*	*Unconjugated estriol multiples of the median in affected pregnancies*
Michigan[1]	26	0.52
Massachusetts[2]	26	0.62*
Texas[3]	22	0.70
Oxford, England[4]	77	0.73
Denmark[5]	42	0.74
Rhode Island/Maine[6]	22	0.79
New York[7]	41	1.00*
Total	256	0.75†

*Ratio of unconjugated estriol in affected to unaffected pregnancies.

†Weighted for the number of affected pregnancies.

Personal communication) but is not included in Table I because it is not yet published.

As a group, the studies yield a median unconjugated estriol of 0.74 multiples of the median for affected pregnancies, with a range of 0.52 to 0.99. This range of values is similar to the range of values reported for α-fetoprotein by various centers,[8] after its discovery as being associated with Down syndrome in 1984. With α-fetoprotein, the assembled data also contained one value from a study that failed to find an association. Ultimately the group consensus was found to be correct, and this is likely to be the case with unconjugated estriol as well.

James E. Haddow, MD, Glenn E. Palomaki, BS, and George J. Knight, PhD
Foundation for Blood Research, Scarborough, ME 04074
Jacob A. Canick, PhD
Brown University, Women and Infant's Hospital, Providence, RI
Nicholas J. Wald and Howard S. Cuckle, DPhil
Medical College of St. Bartholomew's Hospital, London, England

REFERENCES

1. Fisher RA, Suppnick CK, Peabody CT, et al. Maternal serum chorionic gonadotropin, unconjugated estriol and alpha fetoprotein in Down syndrome pregnancies. Am J Hum Genet 1989;45:a259.
2. Osathanondh R, Canick JA, Abell KB, et al. Second trimester screening for trisomy 21. Lancet 1989;2:52.
3. Del Junco D, Greenberg F, Darnule A, et al. Statistical analysis of maternal age, maternal serum alpha-fetoprotein, β-human chorionic gonadotropin, and unconjugated estriol for Down syndrome screening in midtrimester. Am J Hum Genet 1989;45:a257.
4. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum unconjugated oestriol as an antenatal screening test for Down's syndrome. Br J Obstet Gynaecol 1988;95:334-41.
5. Norgaard-Pedersen B, Larsen SO, Arends J, Svenstrup B, Tabor A. Maternal serum markers in screening for Down syndrome. Clin Genet 1990;37:35-43.
6. Canick JA, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Walk NJ. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J Obstet Gynaecol 1988;95:330-3.
7. Macri JN, Kasturi RV, Krantz DA, Cook EJ, Sunderji SG, Larsen JW. Maternal serum Down syndrome screening: unconjugated estriol is not useful. Am J Obstet Gynecol 1990;162:672-3.
8. Wald NJ, Cuckle HS. AFP and age screening for Down's syndrome. Am J Med Genet 1988;31:197-209

Reply

To the Editors: Dr. Haddow et al. call attention to the fact that our published research findings[1] on unconjugated estriol differ from the findings of others.[2-3] It is precisely this fact that prompted our publication. On the basis of a number of studies, the theoretic basis used by Haddow et al. and their research findings deserve close scrutiny before implementation. In a review of references cited by the authors of the letter we find, in fact, support for our caution about the ineffectiveness of unconjugated estriol:

The results suggest that while βhCG levels are independent of the DS ascertainment method, UE levels are low in the [low] AFP group and normal in the [normal] NAFP group. Thus, UE and AFP may not be independent markers in DS pregnancies.

Fisher et al.[4]

This analysis largely confirms the previous statistical analysis of Wald et al.; however, the relative benefit of using unconjugated estriol levels should be studied in greater detail.

Del Junco et al.[5]

The screening performance based on these two markers [MSAFP and hCG] and age does not seem much inferior to that obtainable by including estriol also.

Norgaard-Pedersen et al.[6]

Additional publications on maternal serum unconjugated estriol in Down syndrome screening reveal the following:

If only two of the three biochemical tests are to be used alpha-fetoprotein and human chorionic gonadotropin concentrations is the pair of choice, as alpha-fetoprotein concentration is of separate value in screening for neural tube defects and the performance of alpha-fetoprotein and human chorionic gonadotropin concentrations in combination is not much worse than that of unconjugated oestriol and human chorionic gonadotropin concentrations in combination.

Wald et al.[7]

Our assay results show the SD [standard deviation] of the MSE MoM for the unaffected population to be greater than reported. This means greater overlap of the Down's and unaffected populations and less effectiveness as a screening test.

Swanson[8]

Our model, without the additional measurement of unconjugated estriol levels, appears to be comparable in sensitivity to the analysis proposed by Wald et al.

Petrocik et al.[9]

Canick[10] postulated that, because maternal serum α-fetoprotein was lower in cases of Down syndrome (because of lowered fetal liver production), other markers of fetoplacental origin, specifically unconjugated estriol, should also be lowered. However, Seller[11] has shown that before 20 weeks' gestation the production of α-fetoprotein is not lower in cases of Down syndrome than that in chromosomally normal fetuses. This observation challenges the theoretic basis of the findings of Canick.

In another study[12] from this laboratory, the data show that with the use of certain assays that measure maternal serum unconjugated estriol, marginally higher levels are found in Down syndrome cases compared with controls. Hence unconjugated estriol may not only fail to contribute to detection efficiency of this syndrome, but actually reduce the overall effectiveness of Down syndrome screening. In view of such findings great caution should be exercised before implementation.

Rather than caution, rapid commercialization of a Down syndrome screening protocol including unconjugated estriol is in progress. This is apparent from commercial advertisements that have appeared regularly in the clinical obstetric and genetic literature offering the routine use of unconjugated estriol. During this early developmental stage in Down syndrome screening it is inappropriate to subject clinicians and

patients to the uncertainty about the effectiveness of unconjugated estriol that exists in the biomedical research community. If the very investigators who find low levels of unconjugated estriol are equivocal and skeptical of its use as a marker in Down syndrome screening, patients and physicians should not be pressed to premature human investigation, much less to routine clinical use.

James N. Macri, PhD, Ramana V. Kasturi, BE, MS, David A. Krantz, BS, and Edward J. Cook, PhD
Research Division, NTD Laboratories, Inc., 383 Old Country Road, Carle Place, NY 11514

Shirazali G. Sunderji, MD
Department of Obstetrics and Gynecology, SUNY Health Science Center, 725 Irving Ave., Suite 115, Syracuse, NY 13210

John W. Larsen, Jr., MD
Department of Obstetrics and Gynecology, George Washington University Medical Center, 2150 Pennsylvania Ave., N.W., Washington, DC 20037

REFERENCES

1. Macri JN, Kasturi RV, Krantz DA, Cook EJ, Sunderji SG, Larsen JW. Maternal serum Down syndrome screening: unconjugated estriol is not useful. Am J Obstet Gynecol 1990;162:672-73.
2. Canick JA, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Wald NJ. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J Obstet Gynaecol 1988;95:330-3.
3. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum unconjugated oestriol as an antenatal screening test for Down's syndrome. Br J Obstet Gynaecol 1988;95:334-41.
4. Fisher RA, Suppnick CK, Peabody CT, et al. Maternal serum chorionic gonadotropin, unconjugated estriol and alpha fetoprotein in Down syndrome pregnancies. Am J Hum Genet 1989;45:a259.
5. Del Junco D, Greenberg F, Darnule A, et al. Statistical analysis of maternal age, maternal serum alpha-fetoprotein, β-human chorionic gonadotropin, and unconjugated estriol for Down syndrome screening in mid-trimester. Am J Hum Genet 1989;45:a257.
6. Norgaard-Pedersen B, Larsen SO, Arends J, Svenstrup B, Tabor A. Maternal serum markers in screening for Down syndrome. Clin Genet 1990;37:35-43.
7. Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T. Maternal serum screening for Down's syndrome in early pregnancy. Br Med J 1988;297:883-7.
8. Swanson JR. Maternal serum estriol as an adjunct in screening for Down's syndrome pregnancy. Clin Chem 1989;35:A606.
9. Petrocik E, Wassman R, Kelly JC. Prenatal screening for Down syndrome with maternal serum human chorionic gonadotropin levels. Am J Obstet Gynecol 1989;161:1168-73.
10. Canick JA. Screening for Down syndrome using maternal serum alpha-fetoprotein, unconjugated estriol and hCG. J Clin Immunol 1990;13:30-3.
11. Seller M. Alpha-fetoprotein in midtrimester Down's syndrome fetal serum. J Med Genet 1990;27:240-3.
12. Macri JN, Krantz DA, Kasturi RV, Cook EJ, Larsen JW. Measurement of unconjugated estriol by enzyme-linked immunosorbent assay fails to show an association with Down syndrome [Letter]. Am J Obstet Gynecol 1990;162:1634-5.

Measures of test performance and predictive values

To the Editors:

So all my best is dressing old words new.
Shakespeare
Sonnet 76

In their recent article dealing with the prediction of residual trophoblastic tissue after first-trimester abortion, Schiff et al. (Schiff E, Ben-Baruch G, Moran I, et al. Prediction of residual trophoblastic tissue in first-trimester abortion and low levels of human chorionic gonadotropin β-subunit. Am J Obstet Gynecol 1990;162:797-801) commented on page 800 that "this test has a sensitivity of 86.4%, and a false-negative rate of 3.2% but a specificity of 60.5% and a false-positive rate of 75.9%." It is an elementary fact that the false-negative rate and the true-positive rate (or sensitivity) of a test add up to one (or 100%, depending on whether these rates, which are infact fractions, not true "rates," are expressed as proportions or percentages); the same applies to the false-positive rate and the true-negative rate (or specificity) of a test. Therefore it is self-evident that the above figures cannot be correct.

As if to underscore the need to keep separate in one's mind measures of test performance (true-positive rate, false-negative rate, true-negative rate, and false-positive rate) and predictive values, which I suggested in a previous letter could best be done by thinking of these fractions in terms of conditional probability,[1] these authors have confused their test's false-negative predictive value with the false-negative rate and the test's false-positive predictive value with the false-positive rate. In fact, the false positive rate of their test was 39.5% (60/152); since the sensitivity was 86.4%, the likelihood ratio of the test, which is the true positive rate divided by the false-positive rate, is 2.2. This in turn means that the prior odds on disease (disease, in the present context, refering to the presence of residual trophoblastic tissue in the uterus) are roughly doubled if the test is positive (Posterior odds = Prior odds × Likelihood ratio).

The prior odds on disease in the study were 1:7 (22:152); therefore the posterior odds are 2:7, and the posterior probability of disease 2:9, or approximately 22%. (If O stands for odds, and p stands for probability, then $p = O/1 + O$, and $O = p/1 - p$). Thus, even if we were to intervene for a positive test result (i.e., only if the authors' κ-value was above the designated value), the woman in question would still be four times more likely not to have, than to have, residual tissue in the uterus. Or, to put the matter the other way around, the authors have tried to persuade us that we would intervene unnecessarily less often if we used their test than if we did not. In fact, the opposite is true, given that with a prevalence of residual tissue of only 12.6% no one would adopt a policy of routine curettage for such patients.

That is not to say that the authors' test provides no

diagnostic information, even if that information is of questionable practical value. If the consequences of delaying a necessary curettage were sufficiently detrimental as to warrant curettage in all women (and there is no evidence that it is), then the authors' test would enable one to reduce the number of unnecessary curettages needed to avoid delaying one necessary curettage from 7 to 3 (with delay nonetheless occurring in approximately 14% of cases requiring curettage). This all presupposes, of course, that the authors' method is valid, which it is not, because it is an example of what might be called statistical tautology. They have defined the test using data from 174 patients and then proceeded to test the efficacy of that test on the same 174 patients. Regression toward the mean will always ensure that a given data set will maximize the performance of a test defined on that data set. *Per contra,* the utility of the test when applied to future data sets will always be less. (This is the statistical basis underlying what I believe used to be called the "gee-whiz" phenomenon). One therefore needs no prospective randomized study to deduce that the authors' method will not perform as well in future data sets as it did in the original sample of patients.

Nicholas Kadar, MD

Department of Obstetrics and Gynecology, Albert Einstein Medical Center, Klein Building, Suite 410, 5401 Old York Road, Philadelphia, PA 19141

REFERENCE

1. Kadar N. Specifics of specificity and sensitivity [Letter]. AM J OBSTET GYNECOL 1989;161:1416.

Reply

To the Editors:

Why with the time do I not glance aside to new-found methods and to compounds strange?
Shakespeare
Sonnet 76

We read with interest Dr. Kadar's comments on our article. It seems that there is disagreement regarding the way of calculating the false-positive rate and the false-negative rate. This difference of opinion is also expressed in the letters of Dr. Kadar and Dr. Kolins published in the JOURNAL.[1,2] We prefer to use Dr. Kolins' method by which the false-positive rate is calculated as the percentage of all positive results that were false-positive. In this way the false-positive rate complements the positive predictive value and not the specificity to 1. It seems to us that in this way the disadvantage of the test is more clearly presented. However, since we are dealing with definitions only, which do not change the character of the results, it would be even simpler to represent the false-positive rate in our study as 39.5% instead of 75.9% and this last value as 1 − Positive predictive value.

The statement that curettage is not performed routinely in women such as those described in our study is incorrect. Many physicians are not aware that residual trophoblastic tissue is identified in the curettings in only 13% of these cases. In many centers, as we know, even if one is aware of this fact, it is preferred to perform a curettage, or at least the question arises as to which women should undergo curettage. This is exactly the clinical question we attempted to answer. Therefore, even if the posterior odds ratio is 2:7 and the likelihood ratio of the test is 2.2, a significant percentage (60%) of the curettages could have been prevented. This does not conflict with the fact, also pointed out by us, that there is still a high proportion among the remaining 40% of curettages performed that were unnecessary.

Eyal Schiff, MD, Orit Moran, MD, Gilad Ben-Baruch, MD, and Joseph Menczer, MD

Department of Obstetrics/Gynecology, Sheba Medical Center, Tel-Hashomer, Israel

REFERENCES

1. Kadar N. Specifics of specificity and sensitivity. AM J OBSTET GYNECOL 1989;161:1416.
2. Kolins MD. Reply [Letter]. AM J OBSTET GYNECOL 1989; 161:1416-7.
3. Kolins MD. Definitions of sensitivity, specificity, false negative results, and false positive results. AM J OBSTET GYNECOL 1987;157:517.

Toxicity of vancomycin given during pregnancy

To the Editors: In a recent issue of the JOURNAL Reyes et al. (Reyes MP, Ostrea EM, Cabinian AE, Schmitt C, Rintelmann W. Vancomycin during pregnancy: Does it cause hearing loss or nephrotoxicity in the infant? AM J OBSTET GYNECOL 1989;161:977-81) concluded that vancomycin administration during pregnancy does not produce nephrotoxicity in the newborn infant since plasma creatinine levels were within normal limits at birth. Noteworthy is the fact that it is usually admitted that maternal and fetal plasma creatinine levels are physiologically equilibrated at birth.[1,2] For instance, the comparison of plasma creatinine levels (mean ± SEM) obtained at the time of birth in 16 neonates (7.75 ± 0.83 mg/L) and their mothers (6.67 ± 0.80 mg/L) disclosed a strongly significant relationship ($r = 0.969$; $p < 0.001$) (unpublished data). Subsequently, plasma creatinine levels progressively decline from birth to the fifth day of life in term neonates and to the twenty-first day of life in preterm infants.[1-3] Thus plasma creatinine level at birth is not a reliable marker of glomerular filtration rate,[3] and the study of Reyes et al. does not allow any conclusion to be drawn on vancomycin's nephrotoxicity in newborn infants. Further studies using creatinine or inulin clearances are needed.

J. B. Gouyon, MD, and A. M. Petion, MD

Service de Pédiatrie 2, Hôpital d'Enfants, Bd Mal de Lattre de Tassigny, 21034, Dijon Cedex, France

REFERENCES

1. Rudd TP, Hughes A, Paczek MM, Hodes DT. Reference ranges for plasma creatinine during the first months of life. Arch Dis Child 1983;58:212-5.

2. Feldman H, Guignard JP. Plasma creatinine in the first month of life. Arch Dis Child 1982;57:123-6.
3. Guignard JP. Renal function in the newborn infant. Pediatr Clin North Am 1982;29:777-90.

Reply

To the Editors: We agree with Drs. Gouyon and Petion that plasma creatinine levels of the infant at birth are affected by placental clearance and may be normal. In our study serum creatinine levels were obtained 4 to 6 days after birth in seven of 10 infants born to drug-dependent mothers who received vancomycin during pregnancy. We believe that some elevation of the serum creatinine level would have been evident in these infants at the time of blood sampling if significant renal dysfunction occurred in utero.

Milagros P. Reyes, MD, and Enrique M. Ostrea, Jr., MD
Hutzel Hospital, 4707 St. Antoine Blvd., Detroit, MI 48201

Ethics of new reproductive technologies

To the Editors: The recent discussion of ethics and reproduction by Professor Macnaughton (Mcnaughton M. Ethics and reproduction. AM J OBSTET GYNECOL 1990;162:879-82) deserves additional comment. His opening paragraph discusses our objective as physicians as one of doing good and not harm, and I believe that this is an admirable goal. However, the bulk of his remaining discussion ignores the irreparable harm inflicted on patients from the various reproductive technologies.

Specifically his compilation of deaths as a result of pregnancy-related complications and illegal abortions is not compared to the millions of deaths of human fetuses by sanctioned abortions. Nor does it address the psychological harm suffered by the person undergoing the abortion.

The ethical opinion expressed regarding human embryo research adroitly skirts the issue of life but rather dwells on a concept of personhood to justify this harm. What respect is shown to this life if it is not allowed to complete its potential?

Advances in reproductive technologies that maintain respect for human life should be pursued and applied to the care of our patients. Those that prevent this life from reaching its potential are unworthy of our profession.

Robert J. Blaskiewicz, MD
2821 North Ballas Road, St. Louis, MO 63131

Reply

To the Editors: Professor Blaskiewicz in his letter puts forward the usual arguments of the so-called pro-life lobby. I am not clear how he comes to the conclusion that the new reproductive technologies have inflicted irreparable harm on patients. I can only conclude that he has not been involved himself and really knows nothing about this because my own experience is exactly the opposite.

His arguments against abortion are the usual ones. He seems to be able to tolerate 20% of maternal deaths in the world being due to illegal abortion when we know legal abortion would greatly reduce all this suffering. I consider that quite unacceptable from an ethical point of view. He, of course, must equate the value of a fetus or an embryo above that of the mother and again I cannot agree.

Questions of the origin of life are philsophical and moral ones. Professor Blaskiewicz seems to consider the early embryo to deserve the same respect as a child or an adult. He is entitled to that view, of course, but I do not share it and neither does the British Parliament, which recently voted overwhemlmingly for embryo research to continue under strict control. He also seems to consider that potential life deserves the same respect as actual life. That is similar to saying that because the Prince of Wales is a potential king he must be treated as a king. Patently not true.

I take exception to the implication that those of us involved in new reproductive techniques and in the prevention of maternal death are less caring than the so-called pro-life lobby and I am sure the majority of women have the same view.

Professor Sir Malcolm Macnaughton
University Department of Obstetrics and Gynaecology, Royal Infirmary, Queen Elizabeth Building, 10 Alexandra Parade, Glasgow, Scotland G31 2ER

Twin transfusion syndrome

To the Editors: Shah and Chaffin are absolutely correct to point out the complexities of the diagnosis and management of twin-to-twin transfusion syndrome (Shah DM, Chaffin D. Perinatal outcome in very preterm births with twin-twin transfusion syndrome. AM J OBSTET GYNECOL 1989;161:1111-3). However, I would respectfully suggest that simply an asynchronous hematocrit or birth weight is insufficient evidence on which to base a diagnosis of twin transfusion syndrome, even with monochorial placentation.

I have a special interest in twin transfusion syndrome and would offer the following comments on the basis of my experience. The authors do not discuss how the monochorionicity was confirmed or whether there were injection studies of the fetal circulations of the placentas. This is important because not all monochorial placentas have vascular anastomoses, and without such anastomoses a diagnosis of twin transfusion syndrome seems tenuous. Also, not all discordant growth in monochorial twins is due to twin transfusion. I have seen intertwin discordance of weights of as much as 25%, or even more, which is attributable to asymmetric development of the chorion or asymmetric placental abnormality such as villitis. In addition, the recipient of a twin transfusion may be edematous and be heavier than the co-twin for that reason as well. Thus I have found that linear measurements of infants are often of additional help when comparing intrauterine growth of twins. Similarly, differential hemoglobin values

need to be correlated with the presence and pattern of fetal vascular anastomosis, as well as any evidence of fetal-maternal hemorrhage. Significant fetal-maternal hemorrhage may occur coincidentally with twin transfusion and lead to a significant unilateral anemia of twins. Finally, the authors would do well to differentiate chronic from acute twin transfusion, because the associated pathologic conditions and clinical consequences are different, as are the diagnostic criteria. The chronic twin transfusion syndrome may have helpful ultrasonographic manifestations, although the above additional possibilities must be kept in mind, while acute twin transfusion will not likely be manifested until birth.

Very preterm twins do poorly generally and have an even worse prognosis when truly involved in a twin transfusion. Any intragestational intervention requires accurate diagnosis and can be undertaken only with an awareness of the fact that there is occasional spontaneous subsidence, or even reversal, of twin transfusion. In addition, the source of the disorder is also the source of potential problems with intrauterine therapy, and adequate appreciation of the (as yet) unknowable extent of this potential is required before intervention is undertaken.[1] As Shah and Chaffin pointed out, correlation of the clinical state of the infants must be made with complete details of placental morphologic characteristics before useful interpretations as to the pathophysiologic features can be suggested. In fact, it may take the combined efforts of the placentologist–perinatal pathologist, obstetrician-perinatologist, ultrasonographer–Doppler study expert to sort out the events associated with intertwin vascular anastomosis. It can be a daunting prospect but one that is worthy of detailed assessment considering the seriousness of the condition and suggested interventions.

Virginia J. Baldwin, MD
Autopsy Service, British Columbia's Children's Hospital, 4480 Oak St., Vancouver, British Columbia, Canada V6H 3V4

REFERENCE

1. Baldwin VJ, Wittman BK. Intragestational intervention in twin to twin transfusion syndrome. Pediatr Pathol 1990; 10(pt 1 and 2):79-93.

Reply

To the Editors: We appreciate Dr. Baldwin's interest in our article. The diagnosis of twin-twin transfusion in our series was based on retrospective review of the clinical spectrum, laboratory values, and pathologic examination of the placenta. Although clinical evaluation by neonatologists lends significant credence to such a diagnosis, it is not a measurable parameter. In addition, the literature provides definable criteria for diagnosis, as used in our report. Vascular injection studies were not performed consistently enough to be used for the purpose of study. We accept Dr. Baldwin's criticism about diagnostic criteria but respectfully submit that we are very comfortable with the twin-twin transfusion diagnosis in our series, since it was arrived at after extensive critical review of each case while blinding ourselves to the outcome.

We are not sure what Dr. Baldwin means by asymmetric development of chorion. We would suggest that such a pathologic finding may be a consequence or end result of a chronic in utero twin-twin transfusion. Her suggestion about linear measurements is interesting; however, our experience suggests that linear somatic growth deficiency does occur as a result of severe chronic twin-twin transfusion. All the cases reported in the series, in our opinion, are the result of chronic in utero twin-twin transfusion leading to very preterm delivery. We agree with Dr. Baldwin that acute peripartal twin transfusion does occur and is a consequence of or associated with delivery, but it is not the cause of very preterm delivery as suggested in our series.

We appreciate Dr. Baldwin's support of our contention that consideration of intragestation intervention requires accurate diagnosis. Our experience suggests that spontaneous subsidence or reversal of the twin transfusion process recognized before 28 weeks' gestation must be an extraordinarily rare event. We agree with her that complex problems may be faced in therapeutic attempts. We thank her for supporting what is also our contention: that multifaceted analysis and prenatal evaluation are necessary to understand the pathophysiologic characteristics and that it is a worthy prospect considering the grave consequences of the disorder as supported by our review.

Dinesh M. Shah, MD, and David Chaffin, MD
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, TN 37232-2519

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

Common Problems in Obstetric Care, April 3-6, 1991, Westin Maui, Maui, Hawaii. Sponsored by the Departments of Obstetrics and Gynecology, Cornell University Medical College–The New York Hospital and The University Of Hawaii Medical Center. For information contact: Colette Carmeris, Course Coordinator, Department of Obstetrics and Gynecology, NYH-CUMC, 525 East 68th St., New York, NY 10021. Tel.: (212) 746-3058.

1st International Symposium on AIDS and Reproduction, December 12-15, 1990, Fiera Congress, Genoa, Italy. For information contact: Prof. Francesco Melica, Clinica Ostetrica e Ginecologica dell'Universita, Viale Benedetto XV, 10-16132 Genoa, Italy. Tel.:10-3537708. Fax: 10-3531645.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

آغا خان یونیورسٹی

THE AGA KHAN UNIVERSITY

Faculty of Health Sciences
Medical College

The Aga Khan University is now in its seventh year of operation. Its affiliated Aga Khan University Hospital has well equipped teaching and service laboratories and will have 654 beds when fully operational. The University and the University Hospital are autonomous, privately funded and philanthropic institutions committed to the provision of effective health professional education and health services relevant to Pakistan and the region. Our undergraduate curriculum in medicine stresses integrated training of the basic and clinical sciences with a hands on, problem solving approach to patient care and parallel extensive training in Community Health Sciences.

The Department of Obstetrics and Gynaecology was commissioned in January 1987 and at present there are 6 full-time faculty members working in the Department. The out-patient clinics are heavily scheduled and we are presently doing about 140 deliveries every month. Patient volume and clinical work load are gradually increasing and it is envisaged that there will be 85 Obstetrics and Gynaecology beds at full operation.

We are currently seeking candidates for the following positions:

ASSISTANT PROFESSOR, OBSTETRICS AND GYNAECOLOGY

Candidates applying for this position are required to have completed their postgraduate training in the related field with speciality certification, and must have 3 to 4 years of subsequent clinical and teaching experience.

INSTRUCTOR, OBSTETRICS AND GYNAECOLOGY

Candidates applying for this position are expected to have completed their postgraduate training in Obstetrics and Gynaecology with speciality certification (F.C.P.S. MRCOG, American Boards or equivalent).

Salary and benefits will be offered commensurate with qualifications, experience and level of responsibility. Candidates intending to pursue a career in Pakistan will be preferred. Applications should include detailed résumé, bibliography and names of at least three referees familiar with recent work history. Reply to the **Personnel Director**, The Aga Khan University, P.O. Box 3500, Stadium Road, Karachi-74800, Pakistan.

• Gynecologic Oncologist • Reproductive Endocrinologist
• Clinical Geneticist

The Baystate Medical Center is the major provider of tertiary care in Western Massachusetts. It has recently expanded its resources through its designation as the Western Campus for Tufts University Medical School. In response, the Department of Obstetrics and Gynecology is expanding its full time faculty group.

The Department has over six thousand deliveries and three thousand operative procedures. Our staff consists of forty-five community based physicians, five certified nurse midwives, and eleven full time faculty in the fields of Maternal-Fetal Medicine, Reproductive Endocrinology, Gynecologic Oncology and Primary Care. The Department has a major commitment to Student Education and has an independent Resident Education Program with twenty residents. Excellent opportunities exist for basic and clinical investigation in many areas.

Baystate Medical Center offers a salary and academic rank commensurate with experience. The Department also has a practice plan. Send Curriculum Vitae or contact:

Laurence E. Lundy, MD, Chairman, Obstetrics and Gynecology
John Powell, MD, Chief, Gynecologic Oncology
Patrick O'Grady, MD, Chief, Maternal Fetal Medicine
Halina Wiczyk, MD, Chief, Reproductive Endocrinology

Department of Obstetrics and Gynecology
Baystate Medical Center
780 Chestnut Street
Springfield, MA 01107
(413) 784-5457

We are an equal opportunity employer.

Baystate Medical Center

THE WESTERN CAMPUS OF TUFTS UNIVERSITY SCHOOL OF MEDICINE

A member of Baystate Health Systems

EXCELLENT OPPORTUNITY FOR A BC/BE OB/GYN Physician to join a busy, progressive 8 man practice in the Piedmont Section of North Carolina. **Position open now.** Excellent fringe benefits and salary. New facility with latest equipment. 400 bed hospital 5 minutes away and a teaching hospital with Level III nursery just 20 minutes away. Practice located 1½ hours from mountains, 2 hours from ski slopes, 4 hours from ocean, and 2 large lakes in area. Cultural opportunities available just 20 miles away in a metropolitan area.

Send CV to:
1225 East Garrison Boulevard
Gastonia, NC 28054
Attn: Robert Lester

An Equal Opportunity Employer

OB-GYN

70-Doctor multispecialty group seeking a board certified/eligible Obstetrician-Gynecologist by June 1991 to associate with 4 MDs in an established practice. Well-equipped offices in a modern expanding facility. Big-10 University community of 100,000, superb cultural advantages, ideal for family. Opportunity for medical school affiliation. Excellent salary and benefits with early associateship. Reply with CV:

Ronald H. Deering
101 West University Avenue
Champaign, IL 61820

OBSTETRICS & GYNECOLOGY

Obstetrician/Gynecologist needed to join a five physician department at a major regional multispecialty clinic in Billings, Montana. Clinic setting includes all specialties and is adjacent to an outstanding tertiary care referral hospital. Excellent opportunity for a top notch board eligible or board certified Obstetrician/Gynecologist looking for a challenging practice in a geographic area offering unlimited recreational potential. Those interested send CV to:

John W. Heizer, M.D.,
Medical Director
The Billings Clinic
P.O. Box 35100
Billings, Montana 59107-5100

BRIGHAM AND WOMEN'S HOSPITAL

Obstetrician/Gynecologist

The Brigham and Women's Hospital, a major teaching hospital of Harvard Medical School, seeks several obstetrician/gynecologists to join a busy full-time faculty group practice in general obstetrics and gynecology. The practice is located in a private setting near the hospital.

Applicants should be Board-certified/eligible.

Academic appointment at Harvard Medical School will be granted.

Offers competitive economic package and generous fringe benefits.

Commitment to clinical care and teaching are essential.

Send inquiry and curriculum vitae to:
Kenneth J. Ryan, M.D., Chairman
Department of Obstetrics and Gynecology
Brigham and Women's Hospital
75 Francis Street, ASB1-3-073
Boston, Massachusetts 02115

The Brigham and Women's Hospital is an affirmative action, equal opportunity employer.

CHAIR–OB/GYN

830–bed regional tertiary care, teaching hospital is seeking a Chair for its Department of OB/GYN. The Allentown Hospital–Lehigh Valley Hospital Center has 33 active OB/GYN staff physicians, including a gynecologic oncologist and a reproductive endocrinologist. The current Chair of the Department, who will be leaving at the end of this year, is a perinatologist. The hospital has a fully–approved free–standing OB/GYN Residency Program with two residents each year; it will be making application for a third resident in 1991. The department is also in the process of filling a newly–created position of Program Director of the OB/GYN Residency Program. The hospital trains a total of 70 residents and 400 medical students each year.

The hospital is a regional Level III Perinatal Center. Of over 3,000 births in 1989, 42% were in the high–risk category. The department is supported by a 20–bed Level III NICU and has an active OB/GYN clinic which experienced over 8,000 visits in 1989.

The hospital is a component of HealthEast, a regional, not–for–profit health care system, and is a clinical campus of Hahnemann University in Philadelphia, where the new Chair will hold a vice–chair appointment and an academic appointment at the rank of associate or full professor. The hospital is located in Allentown, a growing metropolitan area in Eastern Pennsylvania, in close proximity to both Philadelphia and New York City.

Candidates must be board–certified physicians with administrative experience and familiarity with both academic and community hospital environments. Interested candidates may write or call for information and send a curriculum vitae, in confidence, to **Headley S. White, Jr., M.D., Sr. Vice President, Medical and Academic Affairs, c/o HealthSearch, 50 College Drive, Suite 323, Allentown, PA 18104, (215) 778–7993.** A descriptive brochure is available.

OBSTETRICAN/GYNECOLOGIST COLUMBIA, SOUTH CAROLINA

A progressive six man OB/GYN Practice with a Maternal-Fetal Medicine Specialist is seeking an additional partner to practice obstetrics and gynecology.

This practice is based at one hospital, has a new office, two ultrasound machines, and in-office laser surgery. We are very active in high risk pregnancies and operative laparoscopy.

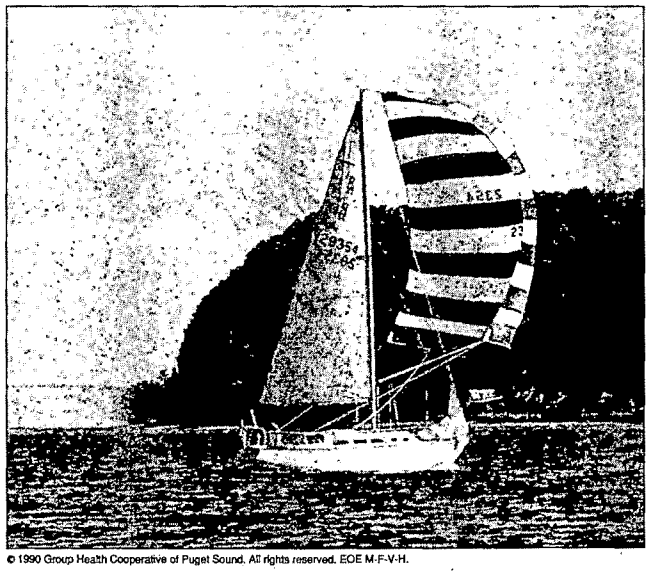
We are looking for a board certified or board eligible, young physician to associate with this practice on a permanent basis. Salary is competitive with comprehensive fringe benefits package.

Association will lead to full and equal partnership. We are seeking a new partner as of July 1, 1991.

For more information, please send a letter and Curriculum Vitae to:

Alexander R. Smythe, II, M.D.
Columbia OB-GYN Associates, P.A.
1333 Taylor St., Suite 5-F
Columbia, South Carolina 29201

Look at health care from our point of view.



© 1990 Group Health Cooperative of Puget Sound. All rights reserved. EOE M-F-V-H.

The obstetrician gynecologists who practice at Group Health are here because quality of life is just as important to them as the quality of their work environment.

They enjoy being part of a highly respected health care organization as much as they like living in one of the top areas in the nation.

If you like our point of view, we invite you to call us toll-free at (800) 543-9323 for more information about career opportunities at Group Health.

Group Health Cooperative
of Puget Sound

Finger Lakes Region
Ithaca, New York

Unique opportunity for patient oriented physician in comprehensive OB-GYN service, building on established patient base of 6,000 patients. To include out-patient gynecology, obstetrics, surgery, infertility, and abortions. First call protection from nurse-midwives. Progressive university community (home of Cornell University) offers cultural attractions of a city with the benefits of small town living. Competitive salary, excellent benefits including malpractice. Summer/Fall 1991.

Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.

Planned Parenthood®
of Tompkins County

ST. LOUIS REGIONAL MEDICAL CENTER
PERINATOLOGIST-GYNECOLOGIST

St. Louis Regional Medical Center, a major teaching hospital of WASHINGTON UNIVERSITY SCHOOL OF MEDICINE in St. Louis, seeks a full time perinatologist and a full time gynecologist to join their faculty.

Applicants should be board certified or active candidates for certification in obstetrics and gynecology. Perinatologist should be board certified or active candidate for certification in maternal-fetal medicine.

Academic rank will be commensurate with experience.

Applicants must be committed to clinical care, teaching, and research.

Letter of interest and curriculum vitae should be sent to:

Klaus J. Staisch, M.D.
Director, Department of Obstetrics and Gynecology
St. Louis Regional Medical Center
5535 Delmar Boulevard
St. Louis, MO 63112

St. Louis Regional Medical Center is an Affirmative action, equal opportunity employer.

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL MILWAUKEE CLINICAL CAMPUS

is seeking applicants for four (4) full-time faculty positions to participate in the development of a new women's health center and academic program.

Sinai Samaritan Medical Center is in the process of consolidating its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department supports a residency program, the largest perinatal service in Wisconsin (5200 deliveries), consultative ultrasound, genetic and infectious disease programs, and reproductive/endocrine services (including an IVF program). The department is affiliated with Planned Parenthood of Wisconsin.

Qualifications include board candidacy or certification in obstetrics and gynecology and a commitment to teaching residents, medical students, and clinical research. Qualifications for the four positions for GENERAL OB/GYN can take into consideration special individual interests. Special expertise of interest for the department are:

- STATEWIDE FAMILY PLANNING AND AMBULATORY CARE (Part time medical director, Planned Parenthood)
- OPERATIVE LAPAROSCOPY, HYSTEROSCOPY
- UROGYNECOLOGY
- RESIDENT/MEDICAL STUDENT EDUCATION
- OB/GYN ULTRASOUND

Private practice opportunities are also available in an associated offsite multispecialty clinic and all faclty members participate in a group practice at the location.

Contact: Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Department of Obstetrics and Gynecology
University of Wisconsin Medical School
Sinai Samaritan Medical Center
Mount Sinai Campus
950 North 12th Street, G405
Milwaukee, WI 53233
(414) 283-7931

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

BC/BE Obstetrician/Gynecologist sought to join small congenial clinic setting within established pre-paid multispecialty group. Practice consists of general obstetrics and gynecology, high risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser are welcomed. Association with resident staff and teaching opportunities available.

Kaiser Permanente offers competitive salaries and excellent benefits including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE

KAISER PERMANENTE
Good People. Good Medicine.

State University of New York
Health Science Center
Syracuse

PELVIC SURGERY FELLOWSHIP

The Department of Obstetrics and Gynecology of S.U.N.Y., Health Science Center at Syracuse is offering a one-year fellowship in Pelvic Surgery for qualified candidates.

The Fellowship will include comprehensive training in benign and malignant pelvic surgery, infertility surgery, laser surgery (abdominal, endoscopic and external genitalia), female urology and endoscopy (laparoscopy, hysteroscopy and colposcopy). The program is designed to provide clinical experience and expertise in gynecologic surgery. Research opportunities are available.

Positions are available beginning July 1, 1991.

Qualifications include ABOG eligible/certified status and eligibility for full NY State licensure.

Requests for applications can be sent to:

Michael S. Baggish, M.D.
Professor and Chairman
Department of OB/GYN
S.U.N.Y., Health Science Center
736 Irving Avenue
Suite 308 West Tower
Syracuse, NY 13210

SUNY, Health Science Center is an AA/EEO Employer

Medical Endocrinologist/ Andrologist

Temple University's Department of OB/GYN is seeking applicants for a full-time faculty position in the Section of Reproductive Endocrinology. This position will be a part of a newly established clinical endocrinology-infertility program. The incumbent will develop a clinical andrology research program in male reproductive physiology and infertility.

Candidates should be Board certified or Board eligible in Medical Endocrinology. Salary and academic rank commensurate with experience. Submit C.V. to: **M. Terry Grody, M.D., Acting Chairman, OB/GYN, Temple University Health Science Center, Broad and Ontario Streets, Philadelphia, PA 19140.**

UNIVERSITY

Choose

TEMPLE

DOCTOR

WE HAVE YOUR LIFESTYLE

Practice in a place you've always wanted to live. Whatever your style, chances are we have the private practice opportunity to match it. After all, with over 80 hospitals in 20 states, we're very well connected. Here are just a few of your options:

Enterprise, AL	**Indianapolis, IN**
Huntsville, AL	**Kansas City, KS**
Montgomery, AL	**Louisa, KY**
Northwest Alabama	**Louisville, KY**
Phoenix, AZ	**Somerset, KY**
Los Angeles Co., CA	**Marksville, LA**
Orange County, CA	**New Orleans, LA**
Denver, CO	**Oakdale, LA**
Central Florida	**Springhill, LA**
Jacksonville, FL	**Las Vegas, NV**
South Florida	**Chattanooga, TN**
St. Petersburg, FL	**San Antonio, TX**
Newnan, GA	**Richmond, VA**
Hoffman Estates, IL	**Greenbrier Valley, WV**

To find out about these and other opportunities, call TOLL-FREE 1-800-626-1590, or send your curriculum vitae to: Manager, Professional Relations, Humana Inc., Dept. QQ-10, 500 West Main Street, Louisville, KY 40201-1438.

Humana®

The department of Obstetrics and Gynecology at the University of Tennessee College of Medicine is actively recruiting a full-time Perinatologist for its Division of Maternal/Fetal Medicine. The teaching hospital serves as the Tertiary Care Center for 80 counties in West Tennessee, East Arkansas, North Mississippi, Southern Missouri and a part of Kentucky; an area with approximately 40,000 deliveries per year. About 50% of the pregnancies delivered at the center are high risk pregnancies. Perinatal services each year include over 20,000 outpatient visits, 7,800 deliveries and over 1,400 patients seen in Reproductive Genetics. Excellent opportunity to participate in teaching in a Board-approved fellowship program and collaborate in ongoing research in clinical Perinatology, Critical Care in Obstetrics, Ultrasound and Prenatal Diagnosis. Board eligibility/certification in Maternal/Fetal Medicine is required. Interested candidates should send curriculum vitae to:

Baha M. Sibai, M.D.
Division of Maternal/Fetal Medicine
University of Tennessee, Memphis
853 Jefferson Avenue, Rm. E-102
Memphis, TN 38103
(901) 528-5660

The University of Tennessee, Memphis is an equal employment opportunity/affirmative action/Title IX/Section 504 employer.

UTMB **University of Texas Medical Branch Galveston, Texas**

The Department of Obstetrics, Gynecology and Reproductive Sciences is currently recruiting for:

Obstetrician-Gynecologist

- Board eligibility or certification in obstetrics and gynecology required.
- Academic rank and salary commensurate with experience and qualifications.

Curriculum Vitae should be sent to:

Garland D. Anderson, M.D.
Jennie Sealy Smith Professor & Chairman
Department of Obstetrics & Gynecology
The University of Texas Medical Branch
Galveston, Texas 77550-2776

UTMB is an equal opportunity M/F/V/H affirmative action employer. UTMB hires only individuals authorized to work in the United States.

The Department of Obstetrics and Gynecology, University of Florida Health Science Center/Jacksonville is currently recruiting for the following faculty positions: Reproductive Endocrinology, Maternal Fetal Medicine (Director and general openings), and a general Obstetrician/Gynecologist. These positions involve patient care, consultative responsibility, teaching, research, and administrative duties. Board certified or eligible to take board required. Academic rank is Assistant/Associate Professor to full Professor. Application deadline: 10/31/90. Anticipated start date: 1/1/91.

UNIVERSITY of FLORIDA

Please submit CV and names/addresses of 3 references to: Robert Thompson, M.D., Associate Chairman, Department of OB/GYN, 655 West 8th Street, Jacksonville, FL 32209.

AAE/EOE

OBSTETRICIAN GYNECOLOGIST

St. Vincent's Medical Center, affiliated with Yale University School of Medicine and New York Medical College, is currently seeking a full-time Board Certified/Eligible Obstetrician Gynecologist to provide general in-patient and out-patient OB/GYN care for hospital service patients. This responsibility will be shared with another full-time Board Certified physician already on staff. The opportunity for limited private practice is available with appropriate space, equipment and support provided.

Excellent opportunity for individual desiring well compensated position with no starting fees or overhead concerns. Full salary with excellent benefits. Excellent hospital facilities with perinatology and neonatology services on site.

Please send curriculum vitae to:

Paul R. Ramirez, M.D.
Chairman
Department of Obstetrics and Gynecology
St. Vincent's Medical Center
2800 Main Street
Bridgeport, Connecticut 06606
(203) 576-5466

KAISER PERMANENTE

OBSTETRICIAN-GYNECOLOGIST

The Permanente Medical Group, Inc., of San Jose, California is looking for BC/BE physicians for immediate openings in the OB/GYN Department. Positions available either at the main facility in San Jose, or at satellite medical offices in Gilroy. The young, dynamic, department is still growing and currently has seventeen physicians and two nurse practitioners. Our outstanding benefits package includes full coverage malpractice insurance. San Jose and Gilroy share excellent access to Bay Area cultural and recreational activities.

Recent salary advances allow extremely competitive compensation starting with the first year of employment.

For more information, please contact:

Jerome L. Nehemiah, M.D.
Chief, Department of Ob/Gyn
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119
or telephone: 408-972-6180 (collect)

Associate Director OB/Gyn for Family Practice Residency

Full-time faculty position available as Associate Director in OB/Gyn training for a 15 resident Family Practice Residency program at a 474-bed community hospital located in Western Pennsylvania approximately 40 miles from Pittsburgh. The residency is a fully accredited program with a faculty presently composed of four board certified family practitioners, one board certified internal medicine physician, one board certified OB/Gyn physician and one behavioral scientist. Successful candidate will have the opportunity to spend half of the time in teaching and the other half in the faculty private practice. OB work generally will be done through residency with private practice emphasis on gynecology.

Salary and benefits are competitive and negotiable. Please call (412) 843-6000, ext. 3017 for more information or send all inquiries along with C.V. to: Director, Family Practice Residency Program, The Medical Center, Beaver, Pa., Inc., 1000 Dutch Ridge Road, Beaver, PA 15009. An equal opportunity employer.

The Medical Center
Beaver, PA

OB/GYN

Free yourself from the business aspects of private practice and enjoy the visibility and advantages of a multi-specialty prepaid group practice in SUBURBAN BOSTON. Experience reasonable work and coverage schedules. As a full-time member of our respected staff, you will work in one of our modern, well-equipped facilities in north suburban or south suburban Boston. Ancillary services are on-site. Locations are within reasonable distance of the ocean, ski resorts, cultural and academic centers. Excellent compensation and fringe benefit package including full malpractice coverage. **Send C.V. to Director of Physician Recruitment, Medical East Community Health Plan, One Worcester Road, Framingham, MA 01701; or call collect (508) 879-0077.**

Medical East™
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

OB/GYN

BUSY, NW PENNA PRACTICE
The only all-female OB/GYN group at 580-bed Saint Vincent Health Center in Erie, PA, this PC has modern offices next to the hospital. In 1989 the 2.5 MD's did 660 deliveries, 200+ major surgeries, 8000+ total procedures/visits, for 11,500 active patients.

Modern Hospital Facility
Tertiary center has 7 LDR's, 1 OB OR. New OB Floor includes ante-partum testing unit, post-partum wing, adjacent GYN beds. Other features: 12-bed Level II NICU; NAACOG certified RN's; excellent equipment includes lasers; air-ground hi-risk transport system; 24-hr anesthesia coverage (includes epidurals).

Enjoy Metro Assets, No Big City Hassles
Erie County's 283,000 population provides for metro culture, schools/colleges, & recreation with an atmosphere of small town friendliness & sense of community. The area has a broad based, growing economy & a wide array of quality, moderately-priced housing.

Great Practice, Better Offer
For two new associates, there'll be an excellent salary, full benefits (6 wks off), & great teamwork environment. Partnership will be offered with/without buy-in after boards & modicum of tenure.

MedQuest Inc.
P.O. Box MQ • Devault, PA 19432-0160
Phone: 215/640-3774 Fax: 215/640-4392

DETAILS WITHOUT OBLIGATION
Call Bill Gregory today (9 AM-9 PM weekdays) or send your CV. You'll get full info with a low-key style even if you just need to chat about job searching in general. We welcome your call.

MRHP

The **Michael Reese Health Plan,** a large well-established HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching. All specialty facilities available.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Alfred Kendrick, M.D.
Medical Affairs
MICHAEL REESE HEALTH PLAN
2545 King Drive, Chicago 60616
or call (312) 808-4551

Michael Reese Health Plan
an equal opportunity employer m/f/h

LAKE ERIE LOCATION
1991 RESIDENTS WELCOME

OB/GYN opportunity in picturesque community of 50,000 with draw area of 180,000. Over 200 beds in modern and progressive hospital located 30 minutes from Ann Arbor, Michigan and 30 minutes from Toledo, Ohio. Beautiful community offers quality lifestyle and easy access to larger cities. Potential for first year of 10-15 deliveries per month and an equal number of Gynecology cases. Send CV or call Greg Flowers, Jackson and Coker, Inc., 115 Perimeter Center Place, Suite 380 AJOGO, Atlanta, GA 30346, Tel. 1-800-544-1987.

Loyola University Chicago
Faculty Positions

Maternal Fetal Medicine

Board Certified/Board eligible physician to join 5 person academic group perinatal practice as a full time faculty member in the Department of Obstetrics and Gynecology.

OB/GYN Generalist

Outstanding opportunity for OB/GYN Board eligible/Board Certified physician who is interested in a full time academic position.

Contact:

John H. Isaacs, M.D., Chairman
Obstetrics and Gynecology Recruitment Committee
Loyola University Chicago
2160 South First Avenue
Maywood, Illinois 60153
(708) 216-3383

Multispecialty clinic in northern Illinois seeks second OB/GYN and fourth family practitioner to join dynamic, high growth practice. Community Health Center Model. New facility. Scholarship and loan repayment available. Paid malpractice and Flexible Compensation Plan. CV to:

John Frana
Executive Director
Crusader Clinic
120 Tay Street
Rockford, IL 61102
(815) 968-0286

EOE

Academic Gynecologic Oncologist

The Dartmouth-Hitchcock Medical Center is searching for a Board Certified Gynecologic Oncologist at the Associate Professor Level. He/She must be an accomplished investigator and be interested in developing a medical center and state-wide academic and clinical program in gynecologic malignancy. Please send CV with names and addresses of three references to:

L. Herbert Maurer, M.D., Chairman
Gynecologic Oncologist Search Committee
Hematology/Oncology
Dartmouth-Hitchcock Medical Center
2 Maynard Street
Hanover, NH 03756

Dartmouth-Hitchcock Medical Center is an Affirmative Action/Equal Opportunity Employer.



Dartmouth-Hitchcock Medical Center

The Hitchcock Clinic
Hanover, New Hampshire

OBSTETRICIAN/ GYNECOLOGISTS

Group Health Association, one of Washington, D.C.'s most respected names in the health maintenance field, has immediate and future openings for board certified or eligible Obstetrician/ Gynecologists.

Consisting of nine multi-specialty medical centers and 200 physicians, Group Health's highly skilled support staff serves over 150,000 members. This staff attends to the details of clinical administration, allowing you the freedom to devote your energy to practicing medicine. Further, GHA's challenging work environment offers a diverse patient population and affiliation with the area's finest hospitals.

A generous benefits package including vacation, sick leave and insurance coverage (malpractice, life, retirement, disability and dental) awaits you in return for your expertise. In addition, you can look forward to company-paid hospital and licensing fees, professional society dues, continuing education as well as predictable office hours.

If you would like more information, please send your C.V. to the address below or call Mary Hines, Physician Recruiter, on 1-800-336-1442 or in DC Metro area, (202) 364-7442.

Turner Bledsoe, M.D., Medical Director

Group Health Association
4301 Connecticut Avenue, N.W.
Washington, D.C. 20008

An Equal Opportunity Employer

ELKO REGIONAL MEDICAL CENTER

762 - 14th Street Elko, Nevada 89801 (702) 738-3111

OBSTETRICIAN/GYNECOLOGIST

Nevada's oldest multi-specialty clinic seeks BE/ BC OB/GYN to join two other OB-GYN's and Nurse Midwife in very busy practice. Lucrative opportunity for full range of specialty practice. All fee for service with excellent collection ratio. Guaranteed salary for six months leading to full partnership. Comprehensive benefit package including malpractice insurance. Elko is a thriving community surrounded by mountains and wilderness areas. Recreation year round. Please send C.V. to:

Cherie Atwood, Administrator
Elko Regional Medical Center
762-14th Street
Elko, Nevada 89801
(702) 738-3111

Fargo Clinic
MeritCare

OB-GYN

Large multispecialty group is seeking BC/BE Obstetrician-Gynecologist to join seven member department. Department is active in donor and husband insemination. Opportunity to develop Invitro and Gift Programs available.

Fargo Clinic is a 250 physician group that practices in 30 locations throughout eastern ND and western MN. The main facility is located next to St. Luke's Hospitals, a 400 bed tertiary care & trauma center. St. Luke's is a NACHRI designated Children's Hospital with a level III ICN.

Fargo-Moorhead is a tri-college community of 130,000 located just minutes from the lake country of western Minnesota. Abundant cultural, recreational, sporting and hunting activities available year around.

Outstanding compensatory package available. Respond to Robert C. Montgomery, M.D., Chairman, Physician Recruitment, 737 Broadway, Fargo, ND 58123.

WORCESTER, MASSACHUSETTS
OB/GYN ASSOCIATE BE/BC

Join Established Busy 2 Doctor Group Affiliated with Major Teaching Hospital/Urban to Rural Lifestyle with Excellent Educational Systems. 3550 square foot medical office connected to the hospital. State of the art facilities including: 12 exam rooms, treatment/ procedure room, and sophisticated ultrasound equipment.

EXCELLENT COMPENSATION PACKAGE INCLUDES:

- Salary
- Malpractice
- Paid CME & Vacation
- Relocation Expenses
- And Much More

Send or FAX CV To:

Judy Goodney
Director of Professional Relations
Central Massachusetts Health Care, Inc.
Mechanics Tower
100 Front Street, Suite 300
Worcester, MA 01608
(508) 798-8667 FAX (508) 798-3069

OB GYN

Part time position available. La Clinica de la Raza, a nonprofit organization in the San Francisco Bay Area, seeks physician to fill the vacancy of OB/GYN. Requirements include current license to practice medicine in the state of California: BC/BE in OB/GYN, current DEA registration. Bilingual English/Spanish or Cantonses preferred. Fee-for-service for all inpatient care. Malpractice insurance provided, excellent fringe benefits.

Send CV or call:
La Clinica de la Raza
Dr. Greene
or the Personnel Office:
1515 Fruitvale Avenue
Oakland, CA 94601
(415) 534-0078

EOE

OB/GYN WISCONSIN

Multi-specialty group in suburban Milwaukee seeks BC/BE Obstetrician/Gynecologist to join 6 physician OB/GYN department. Single site, single hospital practice. Competitive salary and fringe benefits. Forward inquiries and CV to:

Administrator
P.O. Box 427
Menomonee Falls, WI
53051

FACULTY POSITION
DIVISION OF MATERNAL-FETAL MEDICINE
ALBANY MEDICAL COLLEGE

Rapidly expanding division in our Department of Ob/Gyn needs a third board eligible/certified individual. Candidates should have a strong background in obstetric ultrasound as well as a commitment for medical student and resident education. Protected time for research and other academic pursuits guaranteed. New facility nearing completion. Academic rank in accordance with experience. Competitive salary plus attractive faculty incentive plan provided.

Albany, capital of New York State, offers major cultural and recreational opportunities as well as a variety of life styles in a sane environment.

INTERESTED CANDIDATES SHOULD SEND A LETTER OF INTEREST WITH CV TO:

Myron Gordon, M.D.
Chairman, Department of OB/GYN
Albany Medical College A-74
47 New Scotland Avenue
Albany, New York 12208
OR
Paul J. Weinbaum, M.D.
Head, Division of Maternal-Fetal Medicine
Department of OB/GYN A-133

This is a rapidly growing, two-physician OB/GYN practice offering an excellent full- or part-time position for a BC/BE physician. We're located in beautiful Southeastern New Hampshire, where you'll discover the ideal lifestyle. The location offers proximity to mountains, lakes and seacoast, plus a wealth of cultural attractions. It all adds up to a very healthy family environment.

We're offering a competitive salary, and occurrence malpractice premiums are fully paid. What's more, there's an ownership opportunity after the initial employment period. The position will be available by July, 1991, or earlier.

This position has a lot to offer. In a state that has everything.

The group is affiliated with a 330-bed hospital with a Level II Nursery in one of scenic New England's fastest-growing areas, just one hour from Boston.

Reply with CV to: Office Manager, 88 McGregor St., Suite 301, Manchester, NH 03102. (603) 624-8491. This could be everything you always wanted.



COLORADO

BC/BE female/male DO/MD sought to join progressive 2 man group. Association leading to early partnership with excellent income potential. Office located in one of Denver's fastest growing north west suburbs. Opportunity is available July 91 or sooner. Send inquiries and C.V. to:

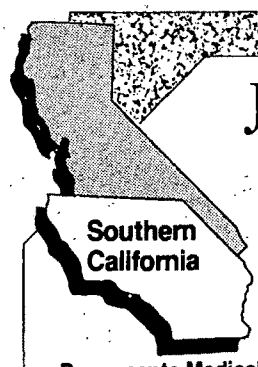
OFFICE OF MEDICAL AFFAIRS Att: A. E. Murray
LUTHERAN MEDICAL CENTER
8300 WEST 38th Avenue
Wheat Ridge, Colorado 80033

Obstetrics/ Gynecology

WISCONSIN: Marshfield Clinic, a multi-specialty group practice with 350 physicians is seeking BE/BC obstetricians/gynecologists to join expanding (40-50 physician) regional centers in Chippewa Falls and Woodruff, Wisconsin. A third OB/GYN is needed for each facility. Chippewa Falls is located 1 1/2 hours from downtown Minneapolis/St. Paul and twenty minutes from a major four year campus of the University of W sconsin. Known as the "Lakeland Area" (over 1,000 navigable lakes) Woodruff is truly one of the most attractive vacation areas in the upper Midwest and renown for its year-round outdoor recreational activities. Both communities offer exceptional recreational, cultural, and educational opportunities. An emphasis on quality practice and life-style is combined with a total compensation package on reaching membership in excess of $200,000. Starting salary negotiable. Fringe benefit package outstanding; includes self-insured malpractice. No start-up expense. Send C.V. and references to:

David L. Draves
Director, Regional Development
1000 North Oak Avenue
Marshfield, Wisconsin 54449
or call collect at
(715)387-5376

Marshfield Clinic



Join The Partnership of Choice

With more than 40 years of successful industry leadership, the **Southern California Permanente Medical Group (SCPMG)** is recognized as the nation's largest and most respected physician-managed, multi-specialty medical group. Our continued growth has created new opportunities for:

CHIEF OF SERVICE
Panorama City, CA

An excellent opportunity for an experienced OB/GYN practitioner to lead an established and growing practice at our Panorama City Medical Center. You'll be responsible for supervising 20 OB/GYN Physicians, a Perinatologist, 8 Certified Nurse Midwives and 4 Nurse Practitioners. This team of professionals will handle approximately 3000 deliveries per year.

CHIEF OF SERVICE
Baldwin Park, CA

An exceptional opportunity to build your own team and participate in the development of our newest 240-bed Medical Center. This facility will have a Level IIA Nursery and a 20-bed NICU with a capacity to handle up to 4000 deliveries per year.

Requirements for Chief of Service positions include:

- Board Certified in OB/GYN
- 5+ years practice experience
- Demonstrated leadership abilities
- Strong interpersonal skills

These are ideal opportunities for a physician who wants to combine a busy clinical practice with day-to-day department management responsibilities.

OBSTETRICIANS & GYNECOLOGISTS

You'll have a choice of professional environments ranging from a 250-bed community hospital to a major teaching facility in a number of prime Southern California locations. Our active practices require proficiency in modern ultrasound and surgical techniques.

SCPMG provides the freedom, technology and resources to focus on quality patient care and the collaborative support of knowledgeable colleagues. **We also provide a highly competitive salary, paid malpractice and predictable hours.**

Call or send your CV to:
Irwin P. Goldstein, M.D., Associate Medical Director
SCPMG, Dept. 006
Walnut Center, Pasadena, CA 91188-8013
(800) 541-7946

KAISER PERMANENTE
Southern California Permanente Medical Group
Partners Practicing Good Medicine

OBSTETRICIAN GYNECOLOGIST

Western Massachusetts staff model Health Maintenance Organization is seeking two full-time, Board Certified/Board Eligible Obstetrician/Gynecologists to join our expert staff of five physicians and five midwives.

Our multi-specialty group practice employs 70 full-time physicians and provides care for a membership of over 65,000 patients in our modern suburban facilities. Inpatient care is provided at a 900-bed Tufts affiliated teaching hospital.

Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118 or call (413) 594-3111.

MedicalWest
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL MILWAUKEE CLINICAL CAMPUS

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE to participate in the development of a new women's health center and academic program. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center is in the process of consolidating its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5200 deliveries), has a dedicated antenatal testing and perinatal ultrasonography (including doppler) unit, and is preparing for an expansion in prenatal diagnostic services (including CVS). The department has a midwifery service and an adolescent pregnancy service. The department has an urban care mission while ready to expand into an up-to-date tertiary care perinatal referral center for Southeastern Wisconsin.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research.

Contact: Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Department of Obstetrics and Gynecology
University of Wisconsin Medical School
Milwaukee Clinical Campus
Sinai Samaritan Medical Center
950 North 12th Street, G405
Milwaukee, WI 53233
(414) 283-7931

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting for positions in our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

REPRODUCTIVE ENDOCRINOLOGIST

• SAN JOSE •

We are currently seeking a Reproductive Endocrinologist to establish and run a reproductive endocrinology subdivision in the department. Duties would include the full spectrum of reproductive endocrinology, outpatient and surgical management of infertility. Fellowship certification would be preferred. The department currently has 17 physicians and is still growing. We offer a generous benefits package including full coverage malpractice insurance. San Jose has beautiful weather and excellent access to Bay Area recreational and cultural activities.

For more information, please contact: Jerome L. Nehemiah, M.D., Chief, Department of OB/GYN, The Permanente Medical Group, Inc., 260 International Circle, Dept. AJOBG, San Jose, CA 95119, or call collect (408) 972-6180. EOE.

KAISER PERMANENTE
Good People. Good Medicine.

OB-GYN

BC/BE OB-GYN sought for full service, 260-bed community hospital in San Francisco's dynamic South of Market area.

- Excellent opportunity to join busy, growing practice.
- Competitive salary and benefits package.

ST. LUKE'S HOSPITAL

Send C.V. to:

Walter Kopp
St. Luke's Hospital
3555 Army Street
San Francisco, CA 94110
(415) 641-6543

OBSTETRICIAN/ GYNECOLOGIST

Immediate opening for a Board Certified/Eligible physician to work with 5 others at a well-established, growing HMO. Rochester Medical Group is a multispecialty medical group practice serving 45,000 patients at multiple sites. Clinical faculty appointment at the University of Rochester is available with this position. Competitive salary/ benefits with opportunities for research and resident teaching. Send resume to:

Patricia Hosking
Rochester Medical Group, PC
1800 Hudson Avenue
Rochester, NY 14617

OB-GYN Physician

Western Massachusetts

("The Heart of the Berkshires")

BC/BE Physician to join thriving 4 physician OB-GYN practice that is part of a 55-physician multi-specialty medical P.C. group. Excellent salary, comprehensive benefits, full malpractice coverage, on call schedule every fourth night, early opportunity to become shareholder. Affiliation is with a modern teaching hospital. Area offerings: Beautiful recreational area; cultural activities include arts/theater (Tanglewood); located near VT, NY, CT borders. Contact Dr. John Buoni at 413-499-8567 or send CV to: Berkshire Physicians & Surgeons, P.C., 44 Charles Street, P.O. Box 4759, Pittsfield, MA 01201.

BRIGHAM AND WOMEN'S HOSPITAL

Perinatologist

The Brigham and Women's Hospital, a major teaching hospital of Harvard Medicel School, seeks a full-time perinatologist to join the Division of Maternal-Fetal Medicine.

Applicants should be board-certified or active candidates for certification in Obstetrics and Gynecology and Maternal-Fetal Medicine.

Academic rank will be commensurate with experience and achievements.

Commitment to clinical care, research, and teaching are essential.

Send inquiry and curriculum vitae to:

Michael F. Greene, M.D.
Director, Division of Maternal-Fetal Medicine
Department of Obstetrics and Gynecology
Brigham and Women's Hospital
75 Francis Street, ASB1-3-078
Boston, Massachusetts 02115

The Brigham and Women's Hospital is an affirmative action, equal opportunity employer.

CLEVELAND CLINIC FOUNDATION

Excellent opportunity available for a Board Certified gynecologist with special interest in colposcopy, education or urogynecology.

Send curriculum vitae to:

Jerome L. Belinson, M.D.
Chairman
Department of Gynecology
9500 Euclid Avenue
Cleveland, Ohio 44195

An equal opportunity/affirmative action employer

OBSTETRICIAN/ GYNECOLOGIST

Ob/Gyn: BC/BE physician wanted to join an excellent practice opportunity in the Southwest. An established growing practice in a new 4,000 sq. ft. office, modern setting, 6 exam rooms, procedure room, 3-physician consulting office, computerized system, plus more.

A competitive guaranteed salary is being offered, with excellent fringe benefits. Includes among others; malpractice insurance and car expense.

Recreational amenities and cultural activities are at your disposal. Reasonable housing costs and access to major metropolitan areas also contribute to an excellent quality of life.

For more information, send your CV to:

Lori Bedford
Jordan/Fairfax
6560 N. Scottsdale Rd.
Suite J-106
Scottsdale, AZ 85253

Or call: (800) 258-9900.

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

Seeking a BC/BE Obstetrician/Gynecologist to provide outpatient care in our Pleasanton medical office building and inpatient care at the Kaiser Hospital/Walnut Creek. Position involves supervision of NPs. Academic affiliation available. Enjoy easy access to the recreational and cultural activities of the San Francisco Bay area. We offer competitive salary and an excellent benefits package including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Patrick Golden, M.D., The Permanente Medical Group, Inc., 5600 Stoneridge Mall Road, Pleasanton, CA 94588. EOE

KAISER PERMANENTE
Good People. Good Medicine.

Life.

It's our life's work.

If your mission in life is to practice the ideals of medicine without administrative demands, we invite your inquiry.

Group Health Plan in St. Louis is a staff model HMO that promotes the ideals of private practice while freeing doctors from the financial baggage of the private practitioner.

Group Health Plan isn't for every physician. Just for those few who want to practice medicine without compromise.

For an interview please talk with the Medical Recruiter at (314) 993-5955. Or write Group Health Plan, 11475 Olde Cabin Road, St. Louis, Mo. 63141. An equal opportunity employer.

GROUP HEALTH PLAN

OBSTETRICIAN/ GYNECOLOGIST

Board certified or board eligible obstetrician/gynecologist to join two member department in multispecialty clinic. Office ultrasound, colposcopy, and cryosurgery available. Adjacent to 94 bed community hospital with special care nursery.

Worthington, MN is a community of 11,000 located in the highly productive corn belt. Recreational activities include good hunting, fishing, and two 18 hole golf courses. Excellent schools, pollution free environment, with low crime rate make this an ideal community for family living.

Excellent compensation package available. Respond to Mr. John Sieve, Administrator, Worthington Medical Center, 508 10th Street, Worthington, MN 56187 or call 507-372-2921.

REPRODUCTIVE ENDOCRINOLOGIST

Position available immediately for Board Certified or eligible Reproductive Endocrinologist. Exceptionally attractive academic position for Junior Faculty at Assistant Professor level. Would join two other Board Certified Reproductive Endocrinologist in a Division with an approved fellowship program, ongoing NIH funded research, and a growing IVF program and private practice. Apply to John E. Buster, M.D., Professor and Director, Division of Reproductive Endcrinology, Department of Obstetrics and Gynecology, University of Tennessee, Memphis, 956 Court Avenue, Room D328, Memphis, TN 38163.

The University of Tennessee, Memphis is an equal employment opportunity/ affirmative action/Title IX/ Section 504 employer.

Ob/Gyn Physician

1,400 square foot new medical office available in beautiful Willamette Falls Health Center in a rapidly growing area near Portland, Oregon. Reserve parking, conference room, immediate access to freeway. Current tenants include Family Practice, Internal Medicine, Dermatology, Immediate Care and Oral Surgery.

Contact Christy Carl at (503) 654-7111.

Faculty Position: Assistant Professor, Department Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco (UCSF).

The Department of Obstetrics, Gynecology, and Reproductive Sciences, UCSF, announces a national search for an obstetr cian-gynecologist with particular expertise in reproductive genetics to be recruited at the assistant professor level. This is a full-time academic appointment meant to add to the academic, investigative, and teaching program of this reproductive genetics unit and the Department. Applicants should be board certified/eligible in Obstetric and Gynecology and board certified/eligible in Medical Genetics.

Responsibilities will include development of an independent research program in some aspect of reproductive genetics, participation in discharging this unit and department and department's clinical responsibilities, and participation in medical student, house staff, and fellow education. Salary commensurate with experience and academic status.

UCSF is an Equal Opportunity Employer. Women and minorities are encouraged to apply. Please send letters of inquires and curriculum vitae to Mitchell S. Golbus, M.D., Chair, Search Committee, U-262, University of California, San Francisco, San Francisco, CA 94143-0720.

Faculty Position: Chief of Obstetrics and Gynecology, University of San Francisco (UCSF)/Mount Zion Medical Center

The Department of Obstetrics, Gynecology and Reproductive Sciences, UCSF, announces a national search for a chief of the UCSF obstetrics and gynecology service at its Mount Zion Hospital and Medical Center. This is a full-time academic appointment with excellent opportunities to develop a new program which is an integral component of the Department.

The Mount Zion department is developing programs in gynecologic urology, general obstetrics and gynecology, a mature women's (menopausal) program, and gynecologic oncology. The University has established Mount Zion as the site for its new Comprehensive Cancer Center, and the John Kerner Endowed Professorship in Gynecologic Oncology has just been established.

Responsibilities will include medical student and house staff education, junior faculty development, development of a clinical practice, and administration. Research space is available for this individual and/or faculty recruitment. Salary commensurate with experience and academic status.

UCSF is an Equal Opportunity Employer. Women and minorities are encouraged to apply. Please send a curriculum vitae to Mitchell Golbus, M.D., Chair, Search Committee, U-262, University of California, San Francisco, San Francisco, CA 94143.

Fellowship In Reproductive Genetics

The Division of Reproductive Genetics, Department of Obstetrics and Gynecology at The University of Tennessee, Memphis, is offering a two- or three-year fellowship in reproductive genetics beginning July 1991. The fellowship program is approved by the American Board of Medical Genetics. Applicants must have completed a residency in obstetrics and gynecology, and eligibility for medical licensure in Tennessee is required. The fellowship is designed to provide a comprehensive experience in clinical genetics (counseling, prenatal diagnosis, amniocentesis, chorionic villus sampling, fetal blood sampling, ultrasonography, dysmorphology, teratology, alpha-fetoprotein screening). The program emphasizes laboratory training and research in cytogenetics, DNA analyses, inborn errors of metabolism, immunogenetics and population genetics. Fellows are actively involved in clinical and laboratory research projects. Applicants should send a curriculum vitae to: UT Medical Group, Employment Office, 66 N. Pauline, Memphis, TN 38105.

Affirmative Action/Equal Opportunity Employer

Gynecologic Oncologist

Philadelphia, Pennsylvania

Progressive, rapidly expanding Division of Gynecologic Oncology located in Center City has full-time academic position available. Candidates must be Board Eligible/Certified gynecologic oncologists. Recruitment at Assistant/Associate Professor level. Excellent clinical and research opportunities available. Competitive salary and benefits program.

Interested persons send curriculum vitae to:

John A. Carlson, Jr., M.D.
Director, Division of
Gynecologic Oncology
Thomas Jefferson University
School of Medicine
Department of Obstetrics
and Gynecology
1025 Walnut Street
Medical College Building-Room 310
Philadelphia, PA 19107

CHAIRPERSON DEPARTMENT OF OBSTETRICS & GYNECOLOGY

The College of Medicine of the University of Arkansas for Medical Sciences is conducting a search for the position of Professor and Chair, Department of Obstetrics & Gynecology. The Department has active clinical divisions of Maternal-Fetal Medicine, Reproductive Endocrinology, and Gynecologic Oncology. Its research component includes three Ph.D. scientists with expertise in biochemistry, molecular biology, and virology. The candidate must possess sound academic credentials and have shown a committment to patient care, education, and administration. Applications and nominations, including a curriculum vitae, should be sent by November 1, 1990 to: Ernest J. Ferris, M.D., Chair, Search Committee for Chair of Ob/Gyn, U.A.M.S., Mail slot #556, 4301 W. Markham, Little Rock, AR 72205.

The University of Arkansas is an Equal Opportunity/Affirmative Action Employer.

Two person OB-GYN group, well established, in prestigious Long Island community. Looking for BC/BE Associate leading toward partnership. University and Community hospital affiliations, in-office sonography, cryo surgery, and colposcopy. Excellent salary, benefits, and future.

Send C.V. to:
Marc Harvey, M.D., F.A.C.O.G.
100 Manetto Hill Road
Suite 306
Plainview, NY 11803

INDEX TO ADVERTISERS

Aga Khan University
Opportunity Available 49A
Albany Medical College
Opportunity Available 58A
Baystate Medical Center
Opportunity Available 49A
Berkshire Physicians & Surgeons
Opportunity Available 61A
Berlex Laboratories, Inc.
Tri-Levlen 36A,37A,38A
Billings Clinic, The
Opportunity Available 50A
Brigham and Women's Hospital
Opportunity Available 50A,61A
Burroughs Wellcome Co.
Zovirax 19A,20A
Central Massachusetts Health Care, Inc.
Opportunity Available 57A
Cleveland Clinic Foundation
Opportunity Available 61A
Columbia OB-GYN Associates, P.A.
Opportunity Available 51A
Corometrics Medical Systems, Inc.
Model 116 ..... 34A,35A
Crusader Clinic
Opportunity Available 56A
Dartmouth-Hitchcock Medical Center
Opportunity Available 56A
E-Z-EM, Inc.
SonoVu US 6A
Elko Regional Medical Center
Opportunity Available 57A
Fargo Clinic
Opportunity Available 57A
Group Health Association
Opportunity Available 57A
Group Health Cooperative of Puget Sound
Opportunity Available 51A
Group Health Plan
Opportunity Available 62A
Harvey, Marc
Opportunity Available 64A
HealthSearch
Opportunity Available 51A
Humana
Opportunity Available 53A
Jackson and Coker, Inc.
Opportunity Available 56A
Loyola University Chicago
Opportunity Available 56A
utheran Medical Center
Opportunity Available 59A
arshfield Clinic
Opportunity Available 59A
ad Johnson Laboratories
Estrace 31A,32A
Nycon 35 ..... 43A,44A
lical Center, The
pportunity Available 55A
ical East
portunity Available 55A
ical West
portunity Available 60A
MedQuest Inc.
Opportunity Available 55A
Michael Reese Health Plan
Opportunity Available 55A
Ortho Pharmaceutical Corporation
Ortho-Novum 7/7/7 ..... Third Cover,Fourth Cover
Osler Institute, The
Review Course 66A
Permanente Medical Group, The
Opportunity Available 52A,54A,60A,62A
Permanente Medical Group, Northern California
Opportunity Available 8A
Planned Parenthood of Tompkins County
Opportunity Available 52A
Rochester Medical Group, PC
Opportunity Available 61A
Searle
Demulen 45A,46A,47A,48A
Southern California Permanente Medical Group
Opportunity Available 59A
St. Vincent's Medical Center
Opportunity Available 54A
Stuart Pharmaceuticals
Cefotan 3A,4A
S.U.N.Y. Health Science Center
Opportunity Available 53A
Syntex Laboratories, Inc.
Femstat Prefill ..... Second Cover,1A,2A
Synarel 39A,40A,41A,42A
Temple University
Opportunity Available 53A
THC
OB Manager 33A
Thomas Jefferson University School of Medicine
Opportunity Available 64A
University of Arkansas
Opportunity Available 64A
University of California, San Francisco
Opportunity Available 63A
University of Florida
Opportunity Available 54A
University of Tennessee, Memphis
Opportunity Available 53A,63A
University of Texas Medical Branch Galveston
Opportunity Available 54A
University of Wisconsin Medical School
Opportunity Available 52A,60A
UT Medical Group
Opportunity Available 64A
W.L. Gore & Associates, Inc.
GORE-TEX 22A,23A
Washington University School of Medicine
Opportunity Available 52A
Worthington Medical Center
Opportunity Available 62A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

The Osler Institute
Obstetrics and Gynecology Review Course
October 22-27, 1990 – Omaha
November 26-December 1, 1990 – Chicago
Now, your course with case-list reviews and mock oral exams
Sponsored by the Osler Institute and Penn State

OBJECTIVES
- Increase basic knowledge and clinical skills in ob-gyn.
- Assist residents and fellows to organize study
- Prepare exam candidates to pass their tests.
- Provide practicing gynecologists with an update

METHODS
- HOME STUDY MATERIALS with questions and answers
- SEMINAR with projection slides and syllabus
- LABORATORY with microscopic slides
- PRACTICE EXAMS with oral and written parts

*"The faculty was outstanding. The most pleasant thing was learning a tremendous amount, not only from world-famous authorities but from people who are relatively unknown as well."**

TOPICS

Clinical Science
Surgical Anatomy
Embryology
Radiology
Genetics
Teratology
Anesthesiology
Antibiotics

Pathology
Vulva
Vagina
Cervix
Endometrium
Myometrium
Ovary
Placenta

Reproductive Endocrinology
Contraception
Infertility
Amenorrhea
Abnormal Bleeding
Androgen
Prolactin
Menopause

Oncology
Chemotherapy
Vulva and Vagina
Cervix
Uterus
Ovary
Trophoblast
Breast

Benign Gynecology
Infectious Diseases
Urinary Retention
Urinary Incontinence
Pediatric Gynecology
Sexual Assault
Ectopic Pregnancy
Endometriosis

Maternal and Fetal Medicine
Pharmacology
Diabetes
Hypertension
Hematology
Antepartum Testing
Premature Labor
Birth Trauma

FACULTY

Linda Brubaker, M.D.
Rush Medical College
Peter Cherouny, M.D.
Penn State University
Phillip Galle, M.D.
Southern Illinois University
Edward Hill, M.D.
Univ. of Calif., San Francisco
Karen Ireland, M.D.
Oregon Health Science Univ.
Allan Jacobs, M.D.
St. Louis University
Paul Kaminski, M.D.
Penn State University
Conley Lacey, M.D.
Scripps Clinic, La Jolla, Calif.
David Plourd, M.D.
Santa Clara Valley Med. Ctr.
Gerald Quirk, M.D.
University of Arkansas
Robert Scully, M.D.
Harvard University
Gary Shangold, M.D.
University of Chicago
Ramona Slupik, M.D.
Northwestern University
Marion Verp, M.D.
University of Chicago
Richard Voet, M.D.
Univ. of Texas, Southwestern
Wendell Wall, M.D.
University of North Dakota
Stan Williams, M.D.
University of Florida

*"Accommodations were comfortable...."**

GOALS AND LOCATION: This course for residents and practicing gynecologists is a comprehensive review and update offered in the spring and fall. The fall course is more clinically oriented. Home study questions are sent upon registration. Your best value is to repeat the course at half price immediately before exams. The October course will be at the Ramada Hotel Central Omaha, 7007 Grover Street, Omaha, Nebraska. Rates will be $69 single – $74 double. The November course will be at the Hilton Inn, 3003 Corporate West Drive, Lisle, Illinois. Rates will be $70 single – $90 double.

*"...and those little extras...."**

LOWEST AIR FARES: Please call toll-free 1-800-548-8185 for group discounts.

*"...the most education for the money."**

CATEGORY 1 C.M.E. AND FEES:
- Penn State designates this continuing medical education activity for **60 credit hours in Category 1** of the Physician's Recognition Award of the American Medical Association.
- This program has been approved for **60 cognates, Formal Learning**, by The American College of Obstetricians and Gynecologists.
- Practicing Gynecologists: $720
- Residents and Fellows: $480
- Repeating course within 3 years: $360
- Case-list reviews and mock oral exams (half hour sessions) $ 70
- Optional extra day Dec. 2 (more questions, answers and pathology lab): $15
- **Add 10% for within 10 days of the course**
- Attendees not in course hotel add $20/da
- A deposit of $50 will reserve your positio

"...home study material was extremely helpfu

CANCELLATIONS: Refunds subject to $ fee, will be made until the seminar beg

*"...remarkably complete and pleasant."**

INFORMATION:
Joseph H. Selliken, Jr., M.D.
The Osler Institute
1094 Dawn Lane, P.O. Box 221
Terre Haute, IN 47802
(800) 356-7537 or (812) 299-56

Limited Enrollment: OBSTETRICS AND GYNECOLOGY REVIEW REGISTRATION

Name _____

Address _____

City/State/Zip _____

Phone _____

Mail today to:

1094 Dawn Lane, Dept. 10
P.O. Box 2218
Terre Haute, IN 47802

For: ☐ October 22-27, 1990 – Omaha
☐ Nov. 26-Dec. 1, 1990 – Chicago
☐ Check enclosed for $ _____
☐ Please send more information

*Comments by participants.

21- and 28-day regimens

ORTHO-NOVUM® 777 Tablets

Smooth Sailing!



norethindrone/ethinyl estradiol

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28 day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

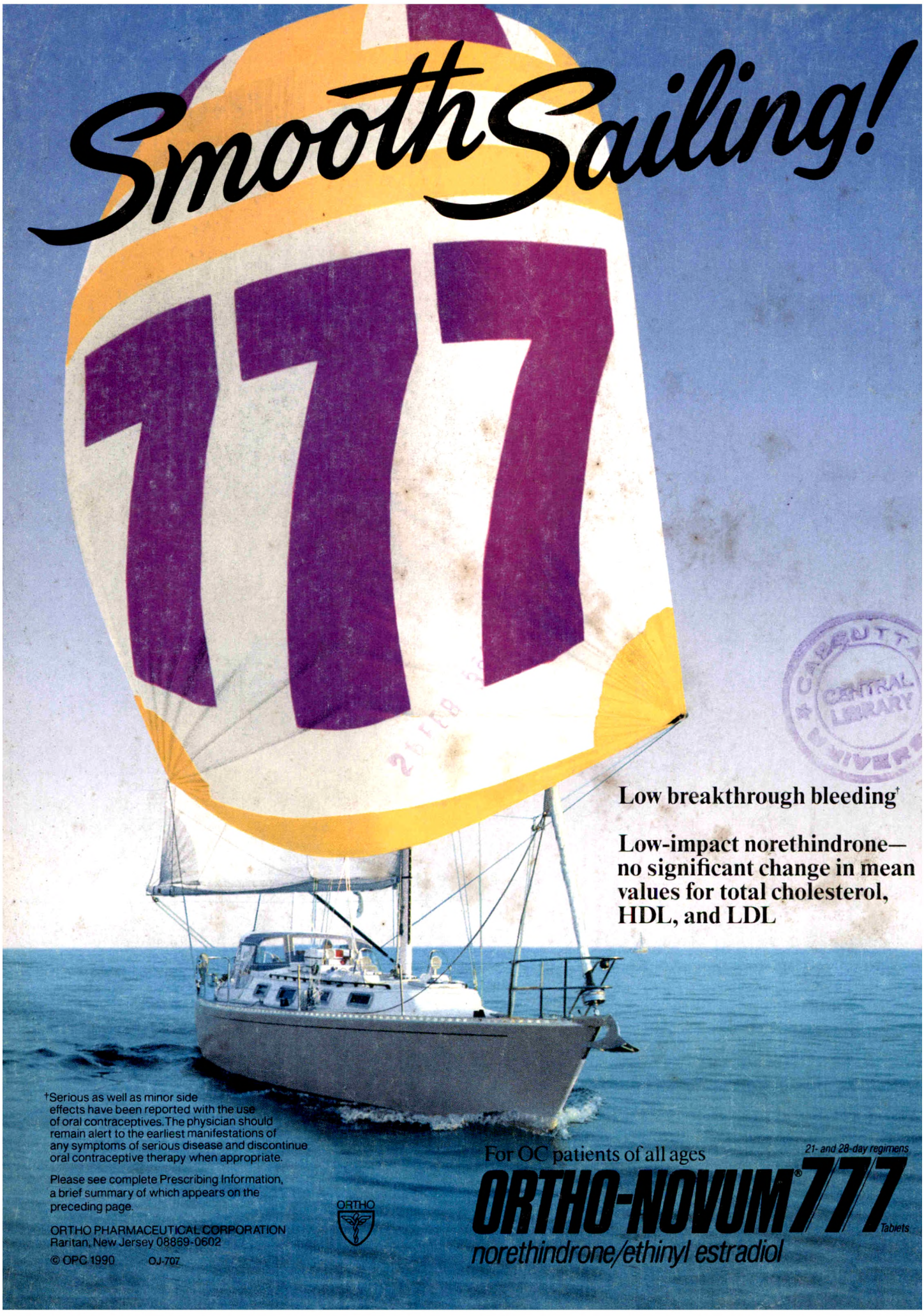
Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

ORTHO PHARMACEUTICAL CORPORATION
Raritan, NJ 08869-0602

ORTHO


Smooth Sailing!
777
Low breakthrough bleeding†
Low-impact norethindrone—no significant change in mean values for total cholesterol, HDL, and LDL
†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.
Please see complete Prescribing Information, a brief summary of which appears on the preceding page.
ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602
© OPC 1990 OJ-707
ORTHO
For OC patients of all ages
21- and 28-day regimens
ORTHO-NOVUM® 777 Tablets
norethindrone/ethinyl estradiol

October **1990**
in two parts, part **2**
volume **163**, *number* **4**

16-4-91

20

SUPPLEMENT TO

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

TOWARD A NEW STANDARD IN ORAL CONTRACEPTION

Proceedings of a symposium held at the
XIII World Congress on Fertility and Sterility

Marrakesh, Morocco
October 3, 1989

Chairmen
Youssef Boutaleb, MD
Joseph W. Goldzieher, MD

These proceedings were supported by a joint educational grant from
SCHERING AG, West Germany, and WYETH-AYERST, United States

Published by
MOSBY-YEAR BOOK, INC.
St. Louis, Missouri 63146-3318

ISSN 0002-9378

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

TOWARD A NEW STANDARD IN ORAL CONTRACEPTION

Proceedings of a symposium held at the
XIII World Congress on Fertility and Sterility

Marrakesh, Morocco
October 3, 1989

Chairmen
Youssef Boutaleb, MD
Professor of Gynecology and Obstetrics
The Medical School
Casablanca, Morocco

Joseph W. Goldzieher, MD
Professor and Director of Endocrine and Metabolic Research
Department of Obstetrics and Gynecology
Baylor College of Medicine
Houston, Texas

These proceedings were supported by a joint educational grant from
SCHERING AG, West Germany, and WYETH-AYERST, United States

Chairmen

Youssef Boutaleb, MD
Professor of Gynecology and Obstetrics
The Medical School
Casablanca, Morocco

Joseph W. Goldzieher, MD
Professor and Director of
Endocrine and Metabolic Research
Department of Obstetrics and Gynecology
Baylor College of Medicine
Houston, Texas

Participants

Michael R. Adams, DVM
Associate Professor, Department of
Comparative Medicine
The Bowman Gray School of Medicine
Wake Forest University
Winston-Salem, North Carolina

Bernd Düsterberg, PhD
Research Laboratories
Schering AG
West Germany

Jan A. Gevers Leuven, MD, PhD
Gaubius Institute TNO
Leiden, The Netherlands

James J. Schlesselman, PhD
Professor, Division of Biostatistics
Department of Preventive Medicine
and Biometrics
F. Edward Hebert School of Medicine
Uniformed Services, University of the
Health Sciences
Bethesda, Maryland

Ulrich Täuber, PhD
Research Laboratories
Schering AG
Berlin, West Germany

Karl Thomas, MD
Professor of Obstetrics and Gynecology
Head, Physiology of Human Reproduction
Research Unit
University of Louvain
Brussels, Belgium

Ian H. Thorneycroft, PhD, MD
John Rock Professor and Vice Chairman
Chief, Reproductive Endocrinology and
Infertility
Department of Obstetrics and Gynecology
Tulane University School of Medicine
New Orleans, Louisiana

Current address
Department of Obstetrics and Gynecology
University of South Alabama
Mobile, Alabama

Mosby Year Book

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright©1990 by Mosby-Year Book, Inc.

Contents

October
Part 2
1990

PART I: CURRENT CONCERNS IN ORAL CONTRACEPTION

Oral contraceptives and breast cancer 1379
James J. Schlesselman, PhD
Bethesda, Maryland

The lifetime risk of breast cancer does not appear to be appreciably affected by the use of oral contraceptives.

Oral contraceptives, lipoproteins, and atherosclerosis 1388
Michael R. Adams, DVM, Thomas B. Clarkson, DVM, Carol A. Shively, PhD, John S. Parks, PhD, and Jay R. Kaplan, PhD
Winston-Salem, North Carolina

Studies in macaques suggest that lowering of high-density lipoprotein cholesterol by progestins is not atherogenic when a sufficiently potent exogenous estrogen is coadministered.

Oral contraceptives and myocardial infarction 1393
Ian H. Thorneycroft, PhD, MD
New Orleans, Louisiana

Epidemiologic data suggest that the risk of myocardial infarction in oral contraceptive users is limited to women over 35 years who smoke.

PART II: A NEW ERA WITH GESTODENE

Clinical acceptability of monophasic gestodene 1398
Bernd Düsterberg and Klaus Brill
Berlin, West Germany

Results of clinical trials of the monophasic low-dose contraceptive containing 30 μg of ethinyl estradiol and 75 μg of gestodene among 100,000 women are reported.

(Contents continued on page 6A)

Vol. 163, No. 4, Part 2, October 1990. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send change of address to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 7351. Annual subscription rates for 1991: domestic, $86.00 for individuals and $150.00 for institutions. Printed in the U.S.A.
Copyright © 1990 by Mosby–Year Book, Inc.

Contents continued from page 5A

Inhibition of ovulation by low-dose monophasic contraceptive containing gestodene. 1404
Karl Thomas, MD, and Leo Vankrieken, BSc
Brussels, Belgium

In 25 subjects a new low-dose monophasic contraceptive containing gestodene provided excellent inhibition of follicular maturation.

Effects of oral contraceptives on lipid metabolism 1410
Jan A. Gevers Leuven, MD, PhD, Marianne C. Dersjant-Roorda, Frans M. Helmerhorst, MD, PhD, Rinse de Boer, MD, Angela Neymeyer-Leloux, MD, and Louis M. Havekes, PhD
Leiden and Hertogenbosch, The Netherlands

Two low-dose combination oral contraceptives containing ethinyl estradiol were shown to have minimal impact on lipid metabolism.

Pharmacokinetics of gestodene and ethinyl estradiol after oral administration of a monophasic contraceptive. 1414
Ulrich Täuber, PhD, Wilhelm Kuhnz, PhD, and Michael Hümpel, PhD
Berlin, West Germany

The pharmacokinetics of a monophasic contraceptive combining gestodene and ethinyl estradiol are described.

1991 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)* Canada	Mexico	Region 1	Region 2	Region 3
Institutions†	$150.00	$182.00	$215.00	$223.00	$238.25	$290.50	$342.50
Individuals‡	$ 86.00	$118.00	$181.00	$159.00	$174.25	$226.50	$278.50
Students, residents‡	$ 40.00	$ 72.00	$105.00	$113.00	$128.25	$180.50	$232.50

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.
*Airmail breakdown—Domestic: First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.
†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.
‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.
Subscriptions may begin at any time.

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 163 *number* 4 *part* 2 OCTOBER 1990

PART I: CURRENT CONCERNS IN ORAL CONTRACEPTION

Oral contraceptives and breast cancer

James J. Schlesselman, PhD

Bethesda, Maryland

Among women in general the risk of breast cancer through 59 years of age does not appear to be affected appreciably by the use of oral contraceptives. Nonetheless, concern continues to be expressed about the effects of early age at first use, long-term duration of use, formulation, and a variety of other factors thought to influence breast cancer risk in the presence of oral contraception. A number of recent studies restricted to young women suggest that long-term use may increase the risk of disease occurring very early, but the present lack of consistent findings in well-conducted epidemiologic studies prevents any certain conclusion with regard to cause-and-effect. However, if an increased risk were indeed present, the most plausible interpretation is that long-term oral contraception promotes earlier clinical manifestation of breast cancer in some women while having no net impact on their lifetime risk of the disease. (AM J OBSTET GYNECOL 1990;163:1379-87.)

Key words: Breast cancer, oral contraception

More than 60 million women throughout the world now use oral contraceptive agents.[1] The two constituents of oral contraceptives, estrogen and progestogen, are known to play a role in both animal and human carcinogenesis.[2-6] There is considerable disagreement, however, about the effects of oral contraceptives on women's risk of breast cancer. Because hormones are considered to be tumor promoters and not initiators,[4] an adverse effect of oral contraception should be revealed by a dose response with duration of use and should be more readily apparent at younger as opposed to older ages of cancer diagnosis.

The findings of 17 different epidemiologic studies reported since 1980 indicate that the risk of breast cancer through ages 55 to 60 years shows no dose response with total duration of use (Fig. 1).[7-23] *Relative risk* in Fig. 1 refers to the risk of breast cancer in users of oral contraceptives divided by the risk in nonusers. A relative risk of 1.3, for example, denotes a 30% increased risk in users; a relative risk of 0.7, on the other hand, denotes a 30% decreased risk in oral contraceptive users, and a relative risk of 1.0 denotes no association between the risk of breast cancer and use of oral contraceptives. There is also no dose response by duration of use before first-term pregnancy (Fig. 2).[24] The epidemiologic findings are based on use of oral contraceptives predominantly between the ages of 25 and 40 years. An emerging consensus is that oral contraception at these ages has no adverse effect on women's risk of breast cancer through middle age.[25]

A number of recent studies, however, suggest that use of oral contraceptives may increase the risk of very early-occurring disease.[26-30] One strongly advocated hypothesis is that long-term use at young ages is the critical dimension of exposure.[28, 30-32] If this were true, then past studies of older women would not show increased risks of breast cancer: (1) because oral contraceptives were not available to older women for use at young ages and (2) because an effect of oral contraceptives might be restricted entirely to early-occurring breast cancer.

Breast cancer in young women

Fig. 3 shows results from nine studies of breast cancer in women under age 45 years that report estimates of relative risk by total duration of oral contraception.[19, 25-30, 33, 34] The relative risks in Fig. 3 tend to be elevated with use beyond 4 years and are uniformly in excess of 1.0 after 8 years' duration. Figs. 4 and 5

From the Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences.
Reprint requests: James J. Schlesselman, PhD, Professor, Division of Biostatistics, Department of Preventive Medicine and Biometrics, F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814.
6/0/21083

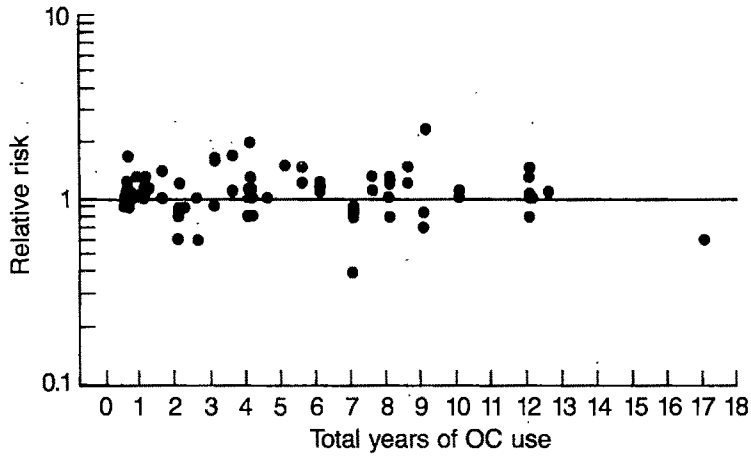


Fig. 1. Breast cancer in women younger than 60 years of age: relative risk by total years of oral contraceptive (OC) use in 17 studies since 1980. (From Schlesselman JJ. Contraception. 1989;40:1-38, Reprinted with permission of Butterworth Publishers.)

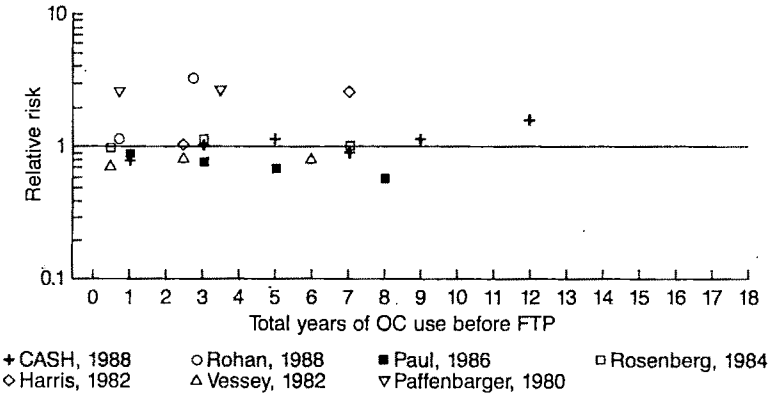


Fig. 2. Breast cancer in women younger than 60 years of age: relative risk by total years of oral contraceptive use before first-term pregnancy. *OC*, Oral contraceptive; *FTP*, first-term pregnancy. (From Schlesselman JJ. Contraception. 1989;40:1-38, Reprinted with permission of Butterworth Publishers.)

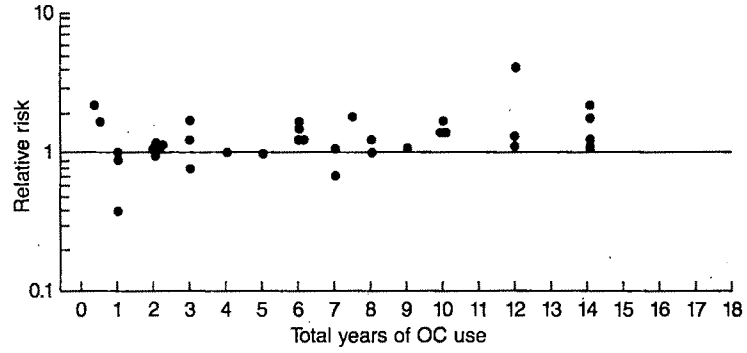


Fig. 3. Breast cancer in women younger than 45 years of age: relative risk by total years of oral contraceptive *(OC)* use in nine studies.

provide study-specific details. The estimates by the United Kingdom National Case-Control Study Group (Fig. 4) reported in May 1989 refer to women under 36 years of age.[30] The study by Pike et al. (Fig. 5) refers to women under 33 years of age.[26]

In 1988 a report from the Royal College of General Practitioners stated that the risk of breast cancer in British women 30 to 34 years of age who had ever used oral contraceptives was significantly increased compared with never users: relative risk = 3.3 (95% con-

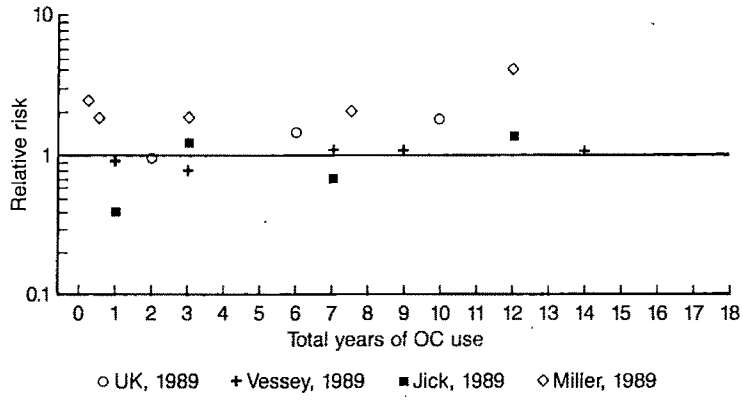


Fig. 4. Breast cancer in women younger than 45 years of age: relative risk by total years of oral contraceptive *(OC)* use.

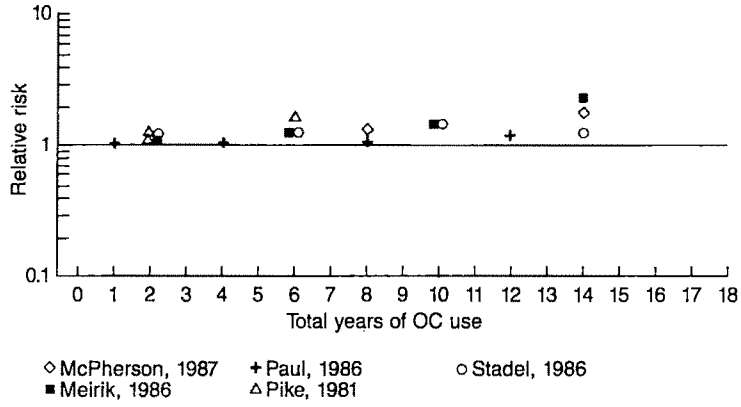


Fig. 5. Breast cancer in women younger than 45 years of age: relative risk by total years of oral coantraceptive *(OC)* use.

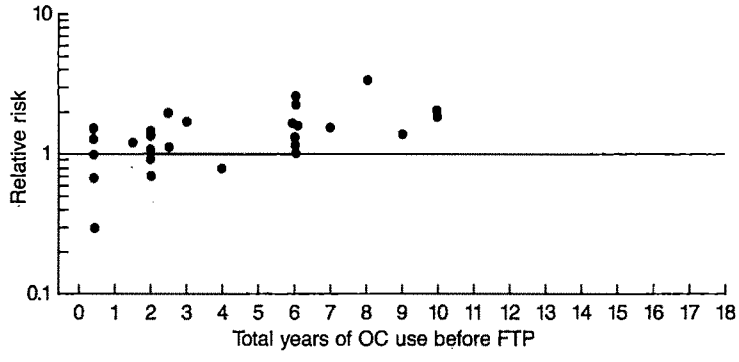


Fig. 6. Breast cancer in women younger than 45 years of age: relative risk by total years of oral contraceptive use before first-term pregnancy in nine studies. *OC*, Oral contraceptive; *FTP*, first-term pregnancy.

fidence interval: 1.3 to 8.8).[22] However, there was no indication of a dose response by duration of use. The risk was also increased in women 25 to 29 years of age who had ever used oral contraceptives, but the estimate (relative risk = 2.5) was very imprecise (95% confidence interval: 0.1 to 52.5) because only six cases of breast cancer were observed in this age range. The risk of breast cancer in women over age 35 years was reported not to be increased for oral contraceptive users. Thus it was suggested that the elevated risk at younger ages for oral contraceptive users might be the result of an accelerated presentation of breast cancer that would otherwise have been diagnosed at a later time.[22]

Fig. 6 shows estimates of the relative risk of breast cancer in women under age 45 years by duration of oral contraceptive use before first-term pregnan-

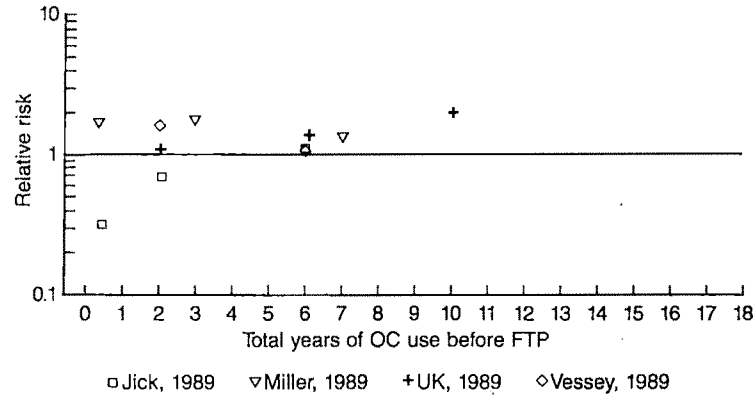


Fig. 7. Breast cancer in women younger than 45 years of age: relative risk by total years of oral contraceptive use before first-term pregnancy. *OC*, Oral contraceptive; *FTP*, first-term pregnancy.

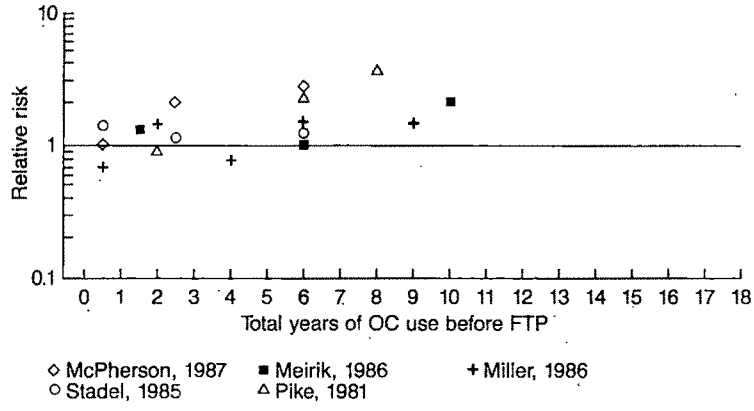


Fig. 8. Breast cancer in women younger than 45 years of age: relative risk by total years of oral contraceptive use before first-term pregnancy. *OC*, Oral contraceptive; *FTP*, first-term pregnancy.

cy.[25-30, 34-36] Because delay of first-term pregnancy itself increases the risk of breast cancer, the relative risks have been adjusted for this factor. The aggregate data suggest a trend of increasing risk with progressively longer periods of oral contraception before first-term pregnancy, with a relative risk of about 1.5 at 4 to 6 years' duration and a relative risk of about 2.0 at 8 to 10 years of use. The evident dose response in Fig. 6 is based on data from nine different studies, only three of which individually show a gradient of risk (United Kingdom, 1989, in Fig. 7; Pike et al., 1981, and McPherson et al., 1987, in Fig. 8).

The Cancer and Steroid Hormone (CASH) Study, which was conducted by the U.S. Centers for Disease Control from 1980 to 1982, shows no effect in young women overall.[35] However, a recent analysis of its data suggests the possibility that prolonged oral contraceptive use in excess of 8 years may accelerate the onset of clinically apparent breast cancer in nulliparous women, particularly those with an early age at menarche.[37, 38] The indication that nulliparous women may be suspectible to an adverse effect of long-term oral contraceptive use is supported by a recent reanalysis of data from the population-based study in Sweden and Norway[39] but not by data from the population-based study in New Zealand (Skegg, DCG. Personal communication, University of Otago, Sept. 6, 1988). The Royal College Study, which also suggests a promotional effect, identifies a different subgroup of women at increased risk—women of parity 1 who use oral contraceptives.[22] The U.K. National Study finds increased risks in both parous and nulliparous women. It also contradicts an earlier study from the United Kingdom in that it reports an effect of oral contraceptive use *after* first-term pregnancy that is just as large as the apparent effect of oral contraceptive use before first-term pregnancy.[30] At present, the absence of any compelling explanation for the striking inconsistencies among these studies prevents any certain conclusion with regard to cause-and-effect.

The rather strong suggestion of an elevated risk of early-occurring breast cancer with long-term oral contraceptive use is not exactly reassuring. One has to be cautious, however, about interpreting studies and find-

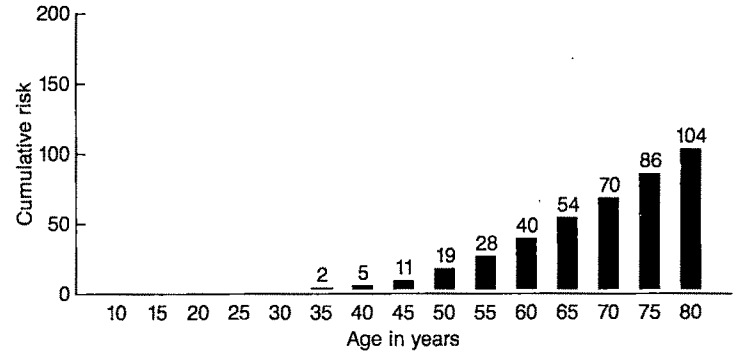


Fig. 9. Breast cancer: cumulative risk per 1000 women, 1981 to 1985, United States.

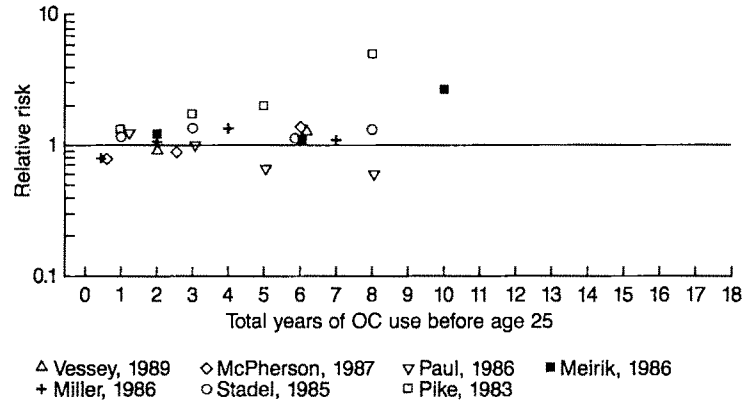


Fig. 10. Breast cancer in women younger than 45 years of age: relative risk by total years of oral contraceptive use before age 25 years.

ings limited to young women, because any alteration in risk found among them may not apply at older ages. Studies of breast cancer in women under age 45 years are restricted to the leading edge of the age-incidence curve. Although the lifetime risk of breast cancer in American women is about 1 in 10,[40] only 1 in 100 (11/1000 in Fig. 9) develops the disease by age 45 years.[41] The recent U.K. National Study was restricted to women under the age of 36 years. The lifetime risk of breast cancer in the United Kingdom is about 1 in 13, but the risk before age 36 years is only about 1 in 500.[42] In the United States the risk of breast cancer before age 36 years is about 1 in 400.[41]

Age at first use, formulation, and latency

Seven studies have presented data on breast cancer risk by duration of oral contraceptive use before 25 years of age.[19, 25, 27, 28, 31, 35, 36] The aggregate data shown in Fig. 10 suggest no dose response, but the findings hint at the possibility that more than 3 years of oral contraceptive use before age 25 years carries a slightly elevated risk of breast cancer. Six of the seven studies report relative risks that exceed 1.0 beyond 3 years of use.

The question whether an early age at first use of oral contraceptives is an important determinant of risk has been addressed in numerous reports. Three population-based studies provide estimates of breast cancer risk by total duration of use in women who started using oral contraceptives before 20 years of age.[19, 30, 35] Fig. 11 shows findings from New Zealand women, 25 to 54 years of age.[19] Fig. 12 shows results in U.S. women, ages 20 to 44 years. Neither study shows evidence of an increased risk of breast cancer in women who started using oral contraceptives at young ages, even in those with long-term use. The U.K. National Study shows a significant dose response by duration of use in women starting oral contraceptives before age 20 years (Fig. 13), but the increased risk is virtually the same regardless of age at first use.[30] Thus duration of use, not age at first use, is the important factor.

Six other studies have reported data on age at first use as a potential breast cancer risk factor.[15, 21, 23, 27, 32, 34] Five of these indicate that age at first use is not important. The one dissenting study[32] was based on interviews that may have been biased.[28, 43]

Effects of oral contraceptive formulation on breast cancer risk have been considered to date in at least 14 different studies reported since 1980.[11-13, 15, 18, 21, 23, 25, 28-31, 34-36, 44, 45] Estimates of risk have

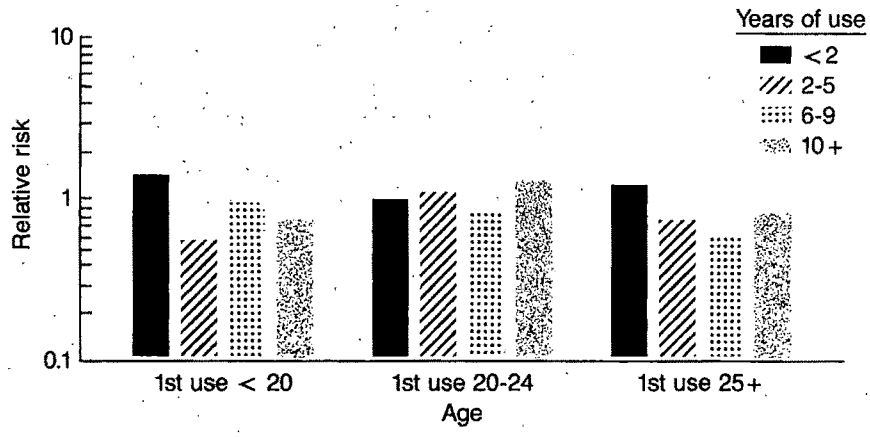


Fig. 11. New Zealand Study: relative risk of breast cancer in women, 25 to 54 years of age, by duration of oral contraceptive use and age at first use.

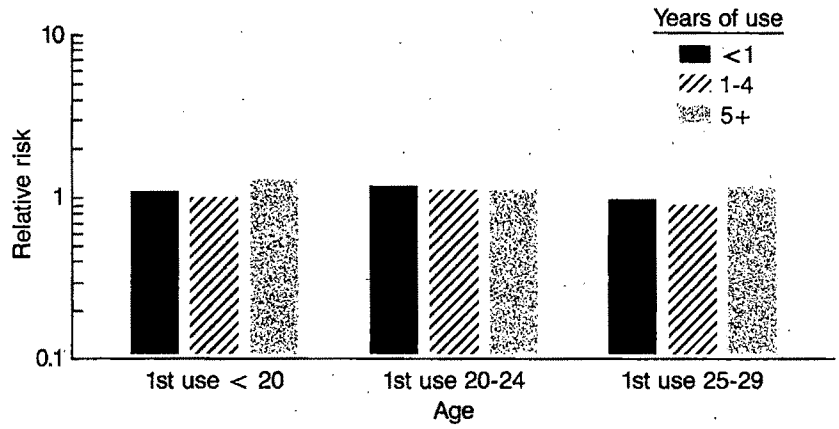


Fig. 12. CASH Study: relative risk of breast cancer in women, 20 to 44 years of age, by duration of oral contraceptive use and age at first use.

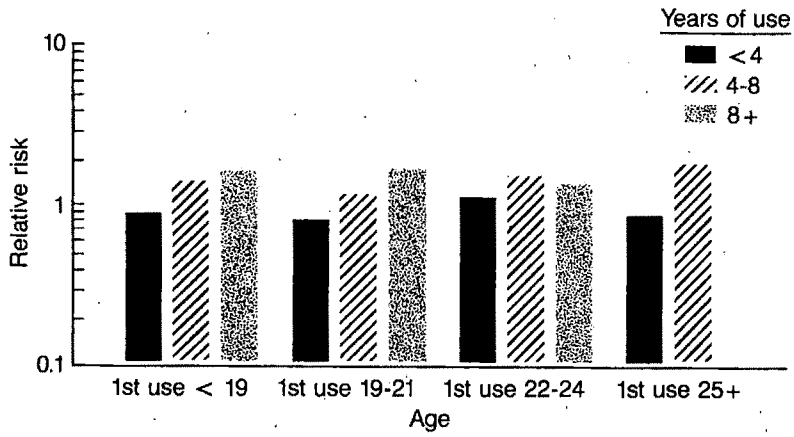


Fig. 13. U.K. National Case-Control Study: relative risk of breast cancer under age 36 years by duration of oral contraceptive use and age at first use.

been given on a brand-specific basis, in relation to various classifications of oral contraceptives, and in relation to the type and total dose of estrogen and progestogen. Suggestions of differential effects have appeared in several reports,[28, 30, 31] but none has been decisively confirmed. To give but one example, consider Fig. 14, which shows breast cancer risk in women under age 45 years by type of estrogen (ethinyl estradiol or mestranol) and duration of use. A significant ($p < 0.01$) dose response in breast cancer risk among women

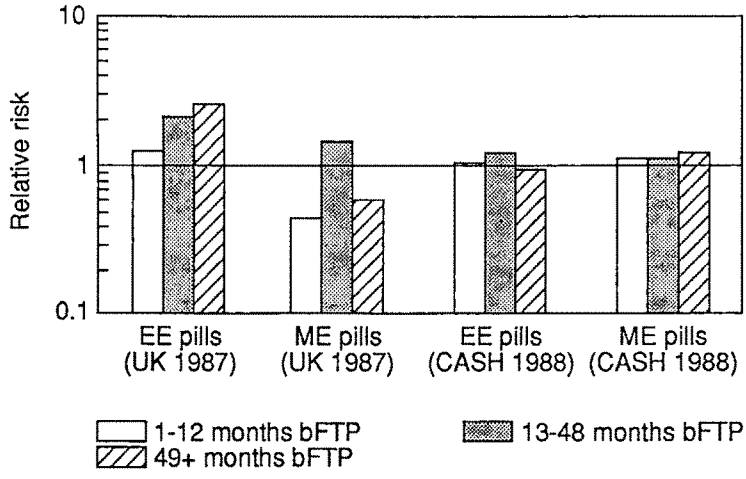


Fig. 14. Relative risk of breast cancer in women younger than 45 years of age by estrogen (*EE*, Ethinyl estradiol; *ME*, mestranol) in combined oral contraceptives used before first-term pregnancy (*bFTP*).

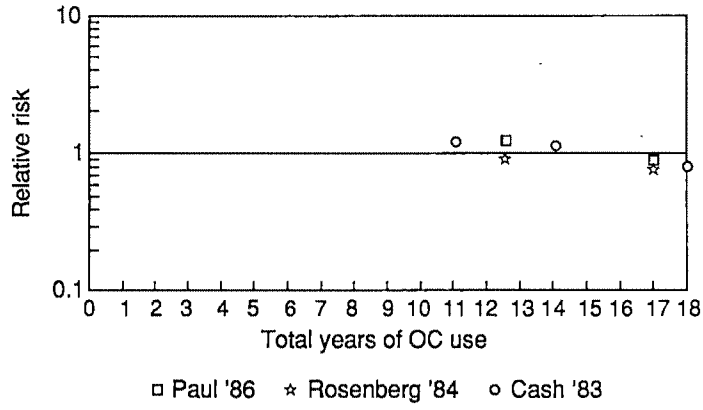


Fig. 15. Breast cancer in women younger than 55 years of age: relative risk by years since first use in women with more than 10 years of oral contraceptive (*OC*) use. (From Schlesselman JJ. Contraception. 1989;40:1-38, Reprinted with permission of Butterworth Publishers.)

using oral contraceptives containing ethinyl estradiol, reported from a study in the United Kingdom, is not supported by data from the CASH Study.

Some investigators have suggested that the "negative" studies can be explained on the basis of a hypothetic long-term latent effect.[46] At least seven reports have addressed this issue directly.[14, 19, 25, 28, 30, 47, 48] None of them, including all the "positive" studies, supports a long-term latency hypothesis.[49, 50] The positive studies only suggest the necessity to accumulate 4 to 8 years of oral contraceptive use before showing an increased risk of early-occurring breast cancer. Fig. 15 shows that in three of the negative studies, no evidence of a latent effect was apparent 10 to 18 years after first use in long-term users.

Studies of oral contraception in women with a family history of breast cancer have reported "positive"[11, 21, 29, 51] and "negative"[13, 14, 17, 20, 52] findings with almost equal frequency. The evidence thus far, which is based on large, well-conducted studies, offers reassurance that women with a family history of breast cancer are not at increased risk of the disease because of oral contraception (Fig. 16). An almost identical situation occurs for benign breast disease, with "positive"[7, 23, 26, 29, 53] and "negative" studies[11, 13, 14, 46] equally divided. Once again, the evidence favors the view that women with *existing* benign breast disease who initiate use of oral contraceptives do not alter their risk of developing breast cancer.[54]

Comment

Results of epidemiologic studies of oral contraceptives and breast cancer are like brush strokes on a canvas. If one stands far enough back from the details, an image seems to appear, whereas careful scrutiny reveals haphazard splashes of color. Such a phenomenon is acceptable in the realm of art, but not in science, where a penetrating gaze should lead to better, not poorer, perception.

The disagreement among even the well-conducted

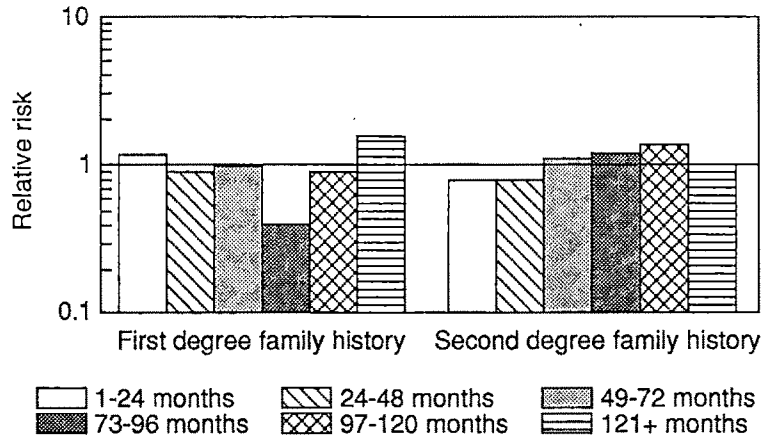


Fig. 16. CASH Study: relative risk of breast cancer in women younger than 55 years of age by total months of oral contraceptive use and family history of breast cancer.

studies suggests that any effect of oral contraceptives on breast cancer risk is likely to be very subtle. This stands in marked contrast to effects of oral contraception on cancers of the endometrium and ovary, in which epidemiologic studies consistently report substantial duration-related protective effects.[24]

Despite the widespread use of oral contraceptives and several reports that suggest increased risks of breast cancer, changes in population-based breast cancer registrations suggest little, if any, effect. In light of the conflicting evidence, regulatory agencies in the United Kingdom and the United States have acted cautiously. In January 1989, an advisory committee to the U.S. Food and Drug Administration recommended no changes in labeling, use, or prescriptions of oral contraceptives. This position was reaffirmed by the Food and Drug Administration later in May 1989 on review of the findings from the U.K. National Case-Control Study.[57] At the same time, the U.K.'s Committee on Safety of Medicines conducted an independent review and, weighing benefits and risks, recommended no change in oral contraceptive prescribing practice on the evidence then available.[58]

I thank Bruce V. Stadel for his comments on the manuscript.

REFERENCES

1. Mishell DR. Contraception. New Engl J Med 1989;320: 777-87.
2. International Agency for Research on Cancer. Sex hormones II. IARC Monogr Eval Carcinog Risks Hum 1979;21.
3. Heywood R. An assessment of the toxicological and carcinogenic hazards of contraceptive steroids. In: Gregoire AT, Blye RT, eds. Contraceptive steroids: pharmacology and safety. New York: Plenum Press, 1986:231-46.
4. Lipsett MB. Steroids and carcinogenesis. In: Gregoire AT, Blye RT, eds. Contraceptive steroids: pharmacology and safety. New York: Plenum Press, 1986:215-29.
5. Henderson BE, Ross R, Berstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res 1988;48:246-53.
6. International Agency for Research on Cancer. Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl 7 1987;296-304.
7. Paffenbarger RS, Kampert JB, Chang HG. Characteristics that predict risk of breast cancer before and after menopause. Am J Epidemiol 1980;112:258-68.
8. Royal College of General Practitioners. Breast cancer and oral contraceptives: findings in Royal College of General Practitioners' study. Br Med J 1981;282:2089-93.
9. Kelsey JL, Fischer DB, Holford TR, et al. Exogenous estrogens and other factors in the epidemiology of breast cancer. J Natl Cancer Inst 1981;67:327-33.
10. Trapido EJ. A prospective study of oral contraceptives and breast cancer. J Natl Cancer Inst 1981;67:1011-5.
11. Brinton LA, Hoover R, Szklo M, Fraumeni JF Jr. Oral contraceptives and breast cancer. Int J Epidemiol 1982; 11:316-22.
12. Harris NV, Weiss NS, Francis AM, Polissar L. Breast cancer in relation to patterns of oral contraceptive use. Am J Epidemiol 1982;116:643-51.
13. Vessey M, Baron J, Doll R, McPherson K, Yeates D. Oral contraceptives and breast cancer: final report of an epidemiological study. Br J Cancer 1983;47:455-62.
14. Rosenberg L, Miller DR, Kaufman DW, et al. Breast cancer and oral contraceptive use. Am J Epidemiol 1984; 119:167-76.
15. Ellery C, MacLennan R, Berry G, Shearman RP. A case-control study of breast cancer in relation to the use of steroid contraceptive agents. Med J Aust 1986;144: 173-6.
16. LaVecchia C, Decarli A, Fasoli M, et al. Oral contraceptives and cancers of the breast and of the female genital tract: interim results from a case-control study. Br J Cancer 1986;54:311-7.
17. Lipnick RJ, Buring JE, Hennekens CH, et al. Oral contraceptives and breast cancer: a prospective cohort study. JAMA 1986;255:58-61.
18. Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. Oral contraceptive use and the risk of breast cancer. N Engl J Med 1986;315: 405-11.
19. Paul C, Skegg DC, Spears GF, Kaldor JM. Oral contraceptives and breast cancer: a national study. Br Med J 1986;293:723-6.

20. Lee NC, Rosero-Bixby L, Oberle MW, Grimaldo C, Whatley AS, Rovira EZ. A case-control study of breast cancer and hormonal contraception in Costa Rica. J Natl Cancer Inst 1987;79:1247-54.
21. Ravnihar B, Zakelj MP, Kosmelj K, Stare J. A case-control study of breast cancer in relation to oral contraceptive use in Slovenia. Neoplasma 1988;35:109-21.
22. Kay CR, Hannaford PC. Oral contraceptives and the pill—a further report from the Royal College of General Practitioners' oral contraception study. Br J Cancer 1988; 58:675-80.
23. Rohan TE, McMichael AJ. Oral contraceptive agents and breast cancer: a population-based case-control study. Med J Aust 1988;149:520-6.
24. Schlesselman JJ. Cancer of the breast and reproductive tract in relation to use of oral contraceptives. Contraception 1989;40:1-38.
25. Vessey MP, McPherson K, Villard-Mackintosh L, Yeates D. Oral contraceptives and breast cancer: latest findings in a large cohort study. Br J Cancer 1989;59:613-7.
26. Pike MC, Henderson BE, Casagrande JT, Rosario I, Gray GE. Oral contraceptive use and early abortion as risk factors for breast cancer in young women. Br J Cancer 1981;43:72-6.
27. Meirik O, Lund E, Adami HO, Bergstrom R, Christoffersen T, Bergsjo P. Oral contraceptive use and breast cancer in young women: a joint national case-control study in Sweden and Norway. Lancet 1986;2:650-4.
28. McPherson K, Vessey MP, Neil A, Doll R, Jones L, Roberts M. Early oral contraceptive use and breast cancer: results of another case-control study. Br J Cancer 1987; 56:653-60.
29. Miller DR, Rosenberg L, Kaufman DW, Stolley PD, Warshauer ME, Shapiro S. Breast cancer before age 45 and oral contraceptive use: new findings. Am J Epidemiol 1989;129:269-80.
30. UK National Case-Control Study Group. Oral contraceptive use and breast cancer in young women. Lancet 1989; 1:973-82.
31. Pike MC, Henderson BE, Krailo MD, Duke A, Roy S. Breast cancer in young women and use of oral contraceptives: possible modifying effect of formulation and age at use. Lancet 1983;2:926-30.
32. Olsson H, Olsson ML, Moller TR, Ranstam J, Holm P. Oral contraceptive use and breast cancer in young women in Sweden. Lancet 1985;1:748-9.
33. Stadel BV, Schlesselman JJ. Oral contraceptives and breast cancer. Lancet 1986;2:922-3.
34. Jick SS, Walker AM, Stergachis A, Jick H. Oral contraceptives and breast cancer. Br J Cancer 1989;59:618-21.
35. Stadel BV, Rubin GL, Webster LA, Schlesselman JJ, Wingo PA. Oral contraceptives and breast cancer in young women. Lancet 1985;2:970-3.
36. Miller DR, Rosenberg L, Kaufman DW, Schottenfeld D, Stolley PD, Shapiro S. Breast cancer risk in relation to early oral contraceptive use. Obstet Gynecol 1986;68: 863-8.
37. Stadel BV, Lai S, Schlesselman JJ, Murray P. Oral contraceptives and premenopausal breast cancer in nulliparous women. Contraception 1988;38:287-99.
38. Stadel BV, Schlesselman JJ, Murray PA. Oral contraceptives and breast cancer. Lancet 1989;1:1257-8.
39. Meirik O, Farley TMM, Lund E, Adami HO, Christoffersen T, Bergsjo P. Breast cancer and oral contraceptives: patterns of risk among parous and nulliparous women—further analysis of the Swedish-Norwegian material. Contraception 1989;39:471-5.
40. Cancer Facts and Figures—1988. Atlanta: American Cancer Society, 1988.
41. National Cancer Institute. Annual cancer statistics review, including cancer trends: 1950-1985. Bethesda: National Institutes of Health, 1988.
42. Anonymous. Cases/controls, breast cancer, and the pill. Lancet 1989;1:1000.
43. Stadel BV, Rubin GL, Wingo PA, Schlesselman JJ. Oral contraceptives and breast cancer in young women. Lancet 1986;1:436.
44. McPherson K, Neil A, Vessey MP, Doll R. Oral contraceptives and breast cancer. Lancet 1983;2:1414-15.
45. Schlesselman JJ, Stadel BV, Murray P, Lai SH. Breast cancer risk in relation to type of estrogen contained in oral contraceptives. Contraception 1987;36:595-613.
46. McPherson K, Coope PA, Vessey MP. Early oral contraceptive use and breast cancer: theoretical effects of latency. J Epidemiol Community Health 1986;40:289-94.
47. Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. Long-term oral contraceptive use and the risk of breast cancer. JAMA 1983;249: 1591-5.
48. Schlesselman JJ, Stadel BV, Murray P, Lai SH. Breast cancer in relation to early use of oral contraceptives: no evidence of a latent effect. JAMA 1988;1:259:1828-33.
49. McPherson K. Latent effect of oral contraceptives on breast cancer. JAMA 1988;260:1240-1.
50. Schlesselman JJ, Stadel BV. In reply—Latent effect of oral contraceptives on breast cancer. JAMA 1988;260: 1241.
51. Black MM, Barclay TH, Polednak A, Kwon CS, Leis HP, Pilnik S. Family history, oral contraceptive usage, and breast cancer. Cancer 1983;51:2147-51.
52. Murray PP, Stadel BV, Schlesselman JJ. Oral contraceptive use in women with a family history of breast cancer. Obstet Gynecol 1989;73:977-83.
53. Lees AW, Burns PE, Grace M. Oral contraceptives and breast disease in premenopausal northern Albertan women. Int J Cancer 1978;22:700-7.
54. Stadel BV, Schlesselman JJ. Oral contraceptive use and the risk of breast cancer in women with a "prior" history of benign breast disease. Am J Epidemiol 1986;123:373-82.
55. Spirtas R. Re: "Breast cancer before age 45 and oral contraceptive use: new findings." Am J Epidemiol 1989;130: 1255-6.
56. U.S. Food & Drug Administration. Summary minutes of the January 5-6, 1989, meeting of the Fertility and Maternal Health Drugs Advisory Committee.
57. U.S. Food & Drug Administration. Statement to the press on May 5, 1989, concerning oral contraceptives and breast cancer.
58. Committee on Safety of Medicines. Oral contraceptives and carcinoma of the breast. Curr Probl No. 26, May 1989.

Oral contraceptives, lipoproteins, and atherosclerosis

Michael R. Adams, DVM, Thomas B. Clarkson, DVM, Carol A. Shively, PhD, John S. Parks, PhD, and Jay R. Kaplan, PhD
Winston-Salem, North Carolina

A nonhuman primate model was developed to study the effects of oral contraceptives on lipoproteins and atherosclerosis. Cynomolgus macaques were selected because of their susceptibility to diet-induced atherosclerosis and because their reproductive physiology, menstrual cycle, and circulating sex hormone patterns are similar to those of human females. The first study compared a vaginal ring containing levonorgestrel and estradiol with an oral contraceptive containing norgestrel and ethinyl estradiol. A second study compared two oral combinations: norgestrel–ethinyl estradiol and ethynodiol diacetate–ethinyl estradiol. As predicted, use of all the contraceptives led to lowering of high-density lipoprotein cholesterol levels. However, contrary to what might be expected, use of the ethinyl estradiol–containing oral contraceptives did not lead to an increase in the prevalence or extent of atherosclerosis. We concluded that ethinyl estradiol neutralized the atherogenic influence of the progestin component of oral contraceptives. (Am J Obstet Gynecol 1990;163:1388-93.)

Key words: Coronary artery atherosclerosis, estrogen, high-density lipoprotein cholesterol, oral contraceptive, progestin

It has been established epidemiologically that diminished plasma concentrations of high-density lipoproteins (HDLs) are associated with increased risk of atherosclerosis and coronary heart disease.[1] Because some oral contraceptives are known to lower HDLs, there has been concern that oral contraceptive use will result in increased atherosclerosis and coronary heart disease.[2] Yet it is not clear whether the epidemiologic relationship can be extended to a situation in which plasma lipid profiles have been altered pharmacologically, such as through the use of oral contraceptives. Furthermore, there is no compelling evidence of a widespread adverse influence of oral contraceptives on coronary heart disease. However, because this remains a controversial subject and is one that is difficult to address experimentally in human beings, we have used a nonhuman primate model of atherosclerosis to study the effects of oral contraceptives on lipoproteins and atherosclerosis.

The cynomolgus macaque *(Macaca fascicularis)* has been used in atherosclerosis research for about 25 years, principally because of its susceptibility to diet-induced atherosclerotic involvement of main branch coronary arteries. We chose it for our research because in addition to its susceptibility to diet-induced atherosclerosis, its reproductive physiology is very similar to that of human beings; the female has a 30-day menstrual cycle and circulating sex hormone patterns that are similar to those of women.[3] Also, a natural menopause occurs in aged macaques.

The macaques selected for the experiments were fed an atherogenic diet that is not very different in nutrient composition from that of the average person who is not following current dietary recommendations relative to coronary heart disease prevention. These diets contained about 40% of calories as fat and 0.1% as cholesterol. A feeding period of 2 to 3 years was needed to produce atherosclerotic plaques in coronary arteries. Total plasma cholesterol concentrations averaged 300 to 400 mg/dl. The extent of atherosclerosis was determined at necropsy and involved perfusion fixation of coronary arteries followed by the collection of five serial sections from each of the three major coronary arteries (Fig. 1).

This line of investigation began several years ago with the finding that premenopausal female macaques share with white women in most Western societies a relative sparing of the coronary arteries in atherosclerosis.[4] As shown in Fig. 2, the extent of atherosclerosis in females, as evidenced by lumen stenosis, was about half that observed in males. Also, as in human beings, plasma HDL cholesterol concentration is lower in males than in females and the ratio of total serum cholesterol to HDL cholesterol is higher, but the gender difference in atherosclerosis persists even after statistical adjustment for this lipid difference. Subsequently, we found that ovariectomy resulted in a loss of this gender difference.[5] Physiologic estrogen replacement in ovariectomized macaques was shown to restore this coronary artery protection.[6]

From the Department of Comparative Medicine, The Bowman Gray School of Medicine, Wake Forest University.
Supported in part by SCOR in Atherosclerosis grant HL-14164 from the National Heart, Lung, and Blood Institute, and contract no. NO1-HD-32800 from the National Institute of Child Health and Human Development.
Reprint requests: Michael R. Adams, DVM, Department of Comparative Medicine, Friedberg Campus, The Bowman Gray School of Medicine, Wake Forest University, 300 So. Hawthorne Rd., Winston-Salem, NC 27103.
6/0/21082

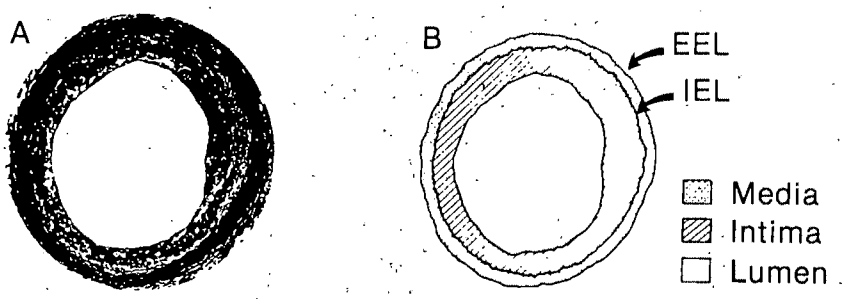


Fig. 1. Extent of atherosclerosis, determined morphometrically, is expressed as the average size of the atherosclerotic plaque as *(A)* evident in the histologic section from a coronary artery and *(B)* indicated by the shaded area in the diagram. *EEL*, External elastic lamina, *IEL*, internal elastic lamina.

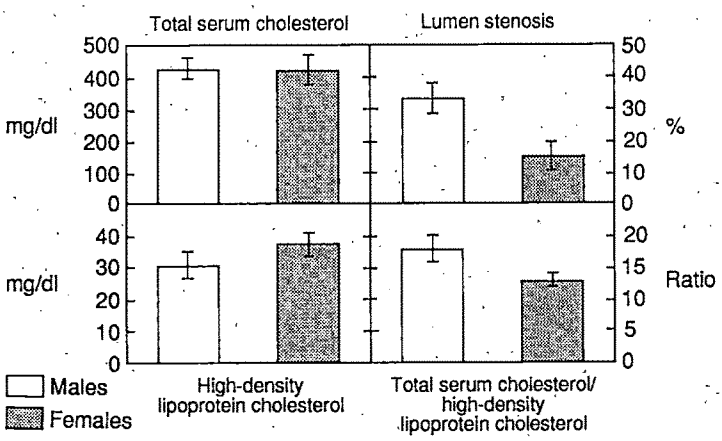


Fig. 2. Serum lipids/lipoprotein profiles and evidence of coronary artery atherosclerosis in male and female cynomolgus macaques. As in human females, macaque females have higher HDL cholesterol and lower total serum cholesterol to HDL cholesterol ratios than do their male counterparts. (Data from Hamm TE Jr, Kaplan JR, Clarkson TB, Bullock BC. Atherosclerosis 1983;48:221-3.)

Oral contraceptive studies

Because endogenous sex hormones and physiologic doses of sex hormones influence atherosclerosis in this species, we also used it to study the effects of contraceptive steroid formulations. We have since completed two studies, which showed that despite these hypothetically atherogenic changes in plasma lipid patterns, oral contraceptive treatment has no adverse influence on coronary artery atherosclerosis.[7,8] We found strong evidence that atherosclerosis was inhibited by combination oral contraceptives containing ethinyl estradiol.

Study 1. In a study reported in 1987, we compared macaques fitted with a vaginal ring containing levonorgestrel and estradiol (n = 13) to others given the oral contraceptive Ovral, norgestrel + ethinyl estradiol (Wyeth-Ayerst, Philadelphia, Pa.) (n = 15) and to untreated controls (n = 15).[7]

Both contraceptive treatments resulted in large reductions in plasma HDL cholesterol concentrations. Neither contraceptive influenced the prevalence of atherosclerosis (percentage of animals with plaque). Animals fitted with the vaginal ring contraceptive showed an increase in the extent of atherosclerosis (plaque area) compared with control animals. In contrast, animals receiving the oral contraceptive had no greater extent of atherosclerosis than did control animals.

These findings suggest that the ethinyl estradiol component of the oral contraceptive neutralized the atherogenic influence of decreased HDL caused by the progestin component. We speculated that estrogen may inhibit the atherosclerotic process through a direct influence on cellular proliferation and metabolism in the arterial intima or through an indirect influence on cellular proliferation or metabolism.

Study 2. Fig. 3 presents the design of the more recent study.[8] A moderately atherogenic diet was fed to 77 female macaques for 31 months.

Methods. During the 7-month preexperimental period, baseline evaluations were made of plasma lipid and lipoprotein variables, after which animals were randomized into three treatment groups by means of a stratification scheme that resulted in equivalence among the groups in regard to pretreatment plasma lipid-risk variables. One group then served as untreated controls, one group was treated with Ovral, and the third group was treated with Demulen, ethynodiol diacetate + ethinyl estradiol (G. D. Searle, Chicago, Ill.).

The oral contraceptives were powdered and mixed

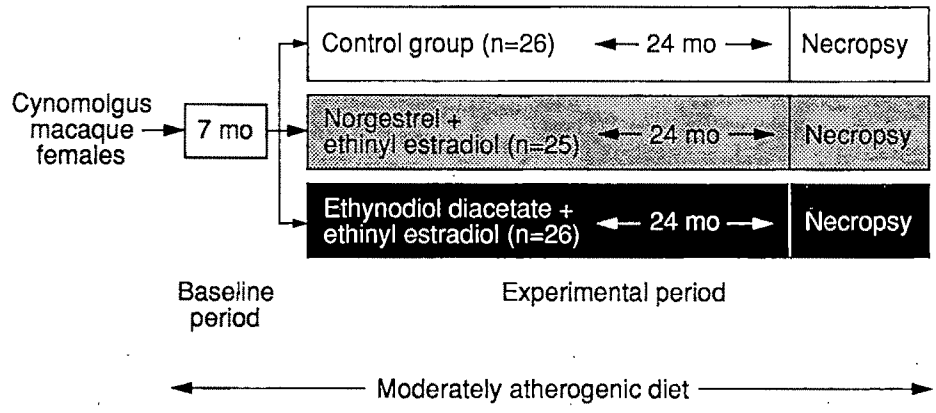


Fig. 3. Design of study to determine the effect of oral contraceptive use on coronary artery atherosclerosis.

Table I. Basis for drug dose

	Cynomolgus macaque		*Woman*	
Average weight (kg)	2.67		50-60	
Calorie consumption (cal/day)	267		1800	
	Progestin	*Estrogen*	*Progestin*	*Estrogen*
Ovral dose, μg	74	7.4	500	50
Ovral dose/100 cal	28	2.8	28	2.8
Demulen dose, μg	148	7.4	1000	50
Demulen dose/100 cal	55	2.8	55	2.8

Table II. Concentrations of progestins in the circulation 3 to 5 hours after feeding measured at 4- to 6-month intervals

Sampling	*Ovral* (ng/ml) (n = 23)*	*Demulen† (ng/ml) (n = 26)*
Mo 5 (baseline)	ND	ND
Mo 2 (treatment)	2.51 ± 0.83	0.95 ± 0.10
Mo 6 (treatment)	2.46 ± 0.33	0.86 ± 0.18
Mo 12 (treatment)	2.96 ± 0.27	1.06 ± 0.10
Mo 18 (treatment)	2.68 ± 0.34	0.79 ± 0.09
Mo 20 (treatment)	3.57 ± 0.35	1.07 ± 0.12

*Measured as major active metabolite (levonorgestrel).

†Measured as major active metabolite ethynodiol diacetate (norethindrone).

in the diet. The macaque dose was determined from the human dose scaled not by body weight but by species differences in daily caloric consumption (Table I). We chose this method because it is a simple way to adjust for differences in basal metabolic rate, which is higher in the monkey, and because our previous study showed that this dosing method resulted in plasma steroid concentrations similar to those of human beings.

Plasma lipids/lipoproteins were measured throughout the experimental period. After 24 months, necropsies were performed to determine the extent of atherosclerosis. Data were analyzed for all animals and for a subset determined to be at high risk because of hyperlipoproteinemia (total serum cholesterol:HDL cholesterol > 4.5). Multiple regression analysis was used to estimate the difference between predicted effects on coronary artery atherosclerosis caused by the risk factor changes induced by contraceptive steroids and observed effects.

Results. Table II summarizes plasma progestin concentrations, either levonorgestrel or norethindrone (the major metabolite of ethynodiol diacetate), 3 to 5 hours after feeding, measured at 4- to 6-month intervals. Concentrations remained relatively stable over the course of the experiment and in a range similar to values found in women using these formulations.

As in human beings, oral contraceptives affected plasma triglyceride concentrations (Fig. 4). As predicted from differences in the estrogen-progestin balance, the macaques treated with the ethynodial diacetate–containing compound had the highest plasma triglyceride concentrations, whereas untreated control animals had the lowest concentrations.

Predictably, total serum cholesterol concentrations were higher in the two treatment groups compared with the control animals (Fig. 5). The effect was most noticeable in the latter half of the treatment period. Also predictably, HDL cholesterol was lowered by both treatments (Fig. 6). This effect was most pronounced in the norgestrel-containing formulation. The ethynodiol diacetate compound was intermediate between the norgestrel compound and untreated controls.

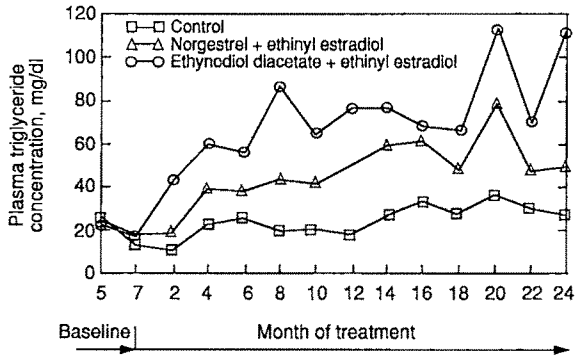


Fig. 4. Plasma triglyceride concentrations by group and month.

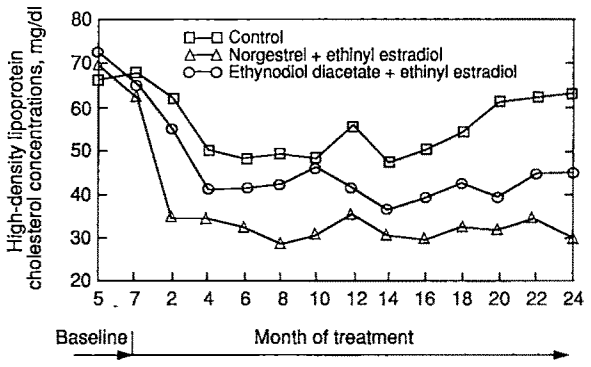


Fig. 6. HDL cholesterol concentrations by group and month.

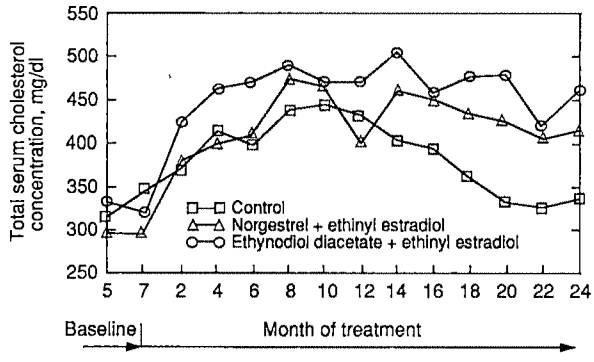


Fig. 5. Total serum cholesterol concentrations by group and month.

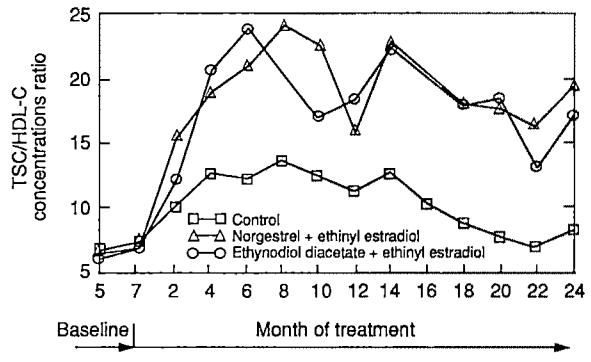


Fig. 7. Ratio of total serum cholesterol concentrations to HDL cholesterol concentrations by group and month.

Table III. Percentage distribution of HDL subfractions of female cynomolgus macaques receiving oral contraceptives

Group	HDL_{2b}	HDL_{2a}	HDL_{3a}	$HDL_{3b,c}$
Control (n = 24)	29.4 ± 4.1	23.2 ± 4.0	27.6 ± 4.4	17.6 ± 3.4
Ovral (n = 23)	5.4 ± 2.5*	23.1 ± 4.7	29.1 ± 4.4	41.4 ± 5.4*
Demulen (n = 25)	12.0 ± 3.2*	19.6 ± 3.0	33.5 ± 4.1	33.2 ± 3.6*

From Parks JS, Pelkey SJ, Babiak J, Clarkson TB. Contraceptive steroid effects on lipids and lipoproteins in cynomolgus monkeys. Arteriosclerosis 1989;9:261-8. By permission of the American Heart Association, Inc.

The ratio of total serum cholesterol to HDL cholesterol, which is a better predictor of extent of coronary artery atherosclerosis than either alone, was similar in the two treatment groups (Fig. 7). Both treatment groups showed markedly elevated total serum cholesterol to HDL cholesterol ratios compared with controls.

Because it is now thought that the HDL_2 fraction is the component of HDL that provides protection against atherosclerosis, we looked at the HDL heterogeneity or the subfractions and distributions of HDL (Table III). The marked decreases in HDL cholesterol observed in both treatment groups can be largely explained by marked decreases in the HDL_{2b} subfractions. From the lipid and lipoprotein data compiled, we concluded that the changes induced by oral contraceptives were in a direction that would be predicted to have an adverse influence on risk of atherosclerotic coronary heart disease.

The concern that adverse influences of oral contraceptives on coronary heart disease might be magnified in women who are at the highest risk because of adverse plasma lipid profiles led us to determine the effects of treatment in all the macaques and also in a subset (n = 34) considered to be at highest risk because of ratios of total serum cholesterol to HDL cholesterol that were greater than 4.5. In macaques as in humans, risk dramatically increases above this ratio.

Fig. 8 summarizes the effect of treatment on extent of coronary atherosclerosis expressed as plaque size. Coronary intimal area refers to the actual plaque size

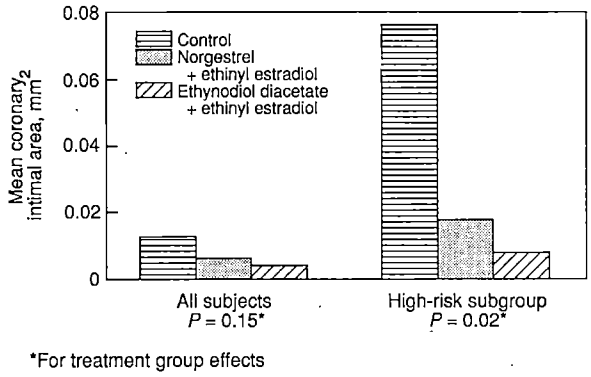


Fig. 8. Effect of treatment on extent of coronary artery atherosclerosis expressed as plaque size. (From Clarkson TB, Adams MR, Shively CA, Koritnik DR. Proceedings of the Consensus Development Conference on Progestogens. Reevaluation of lipid effects on atherosclerosis. Int Proc J 1989;1: 262-6.)

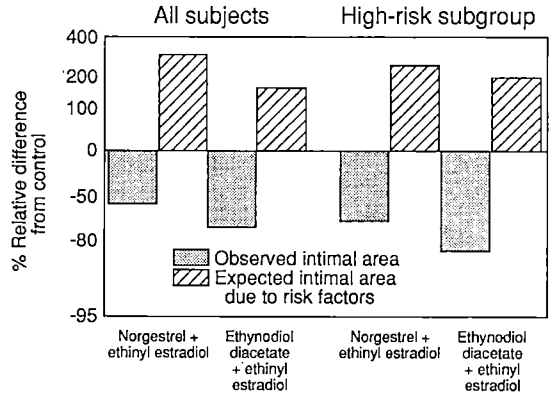


Fig. 9. Observed and expected effects of oral contraception on plasma lipids.

in square millimeters. In both groups, all subjects and the high-risk subgroup, macaques receiving oral contraceptives were less affected than control animals. This difference approached statistical significance in the all-subjects group and was statistically significant among the high-risk animals. There appears to be a large positive or protective effect of oral contraceptives with respect to coronary artery atherosclerosis.

These comparisons did not take into consideration the potential confounding effects of plasma lipid concentrations. Using multiple regression, we were able to predict the effects of oral contraceptives based on changes in plasma lipid levels (Fig. 9). Among all subjects, an approximately twofold increase in extent of atherosclerosis was predicted with each contraceptive formulation. What was observed was a 50% to 75% decrease in extent of atherosclerosis. Among high-risk subjects, the contrast was even more striking. Again, a twofold increase was expected. Instead, a 75% to 85% decrease in extent of atherosclerosis was observed.

Comment

In our studies we found no evidence that combination oral contraceptives have an adverse influence on atherogenesis. In fact, there is strong evidence that oral contraceptives inhibit atherosclerosis, even in the face of marked reductions in plasma HDL cholesterol and especially among high-risk persons. There appears to be a large positive or protective effect of oral contraceptives with respect to coronary artery atherosclerosis.

These studies were done with older, high-dose formulations not in widespread current use. Whether similar effects will be observed with newer, low-dose formulations is unclear. It seems, however, that the estrogen-progestin balance may be important in the protective effect, and although the estrogen dose has decreased in the newer formulations, the progestin dose has decreased proportionally or proportionally more than the estrogen dose. Thus in general the estrogen-progestin balance has not been substantially altered in the new formulations.

Although it remains unknown whether oral contraceptives have similar arterial effects in human beings as in monkeys, the results of our studies are consistent with those of epidemiologic studies of human beings. Although these compounds have been in widespread use for about 25 years, there is no compelling evidence of a widespread adverse influence on coronary heart disease risk. On the contrary, the incidence of coronary heart disease among women in the United States has declined markedly over this same time period. Also, although there is a well-known increase in risk associated with current oral contraceptive use, the increase is very small and not in proportion to effects on plasma lipoproteins.[9] In addition, current evidence indicates that this increase in coronary heart disease risk is largely confined to users of older, high-dose estrogen-progestin formulations who are cigarette smokers.[9] Also, the increased risk disappears after cessation of oral contraceptives[9] thus suggesting a nonatherogenic mechanism such as thrombosis or vasospasm. Furthermore, some studies actually suggest a decrease in the risk of coronary heart disease in past users of oral contraceptives.[9, 10] Taken together, these findings indicate that oral contraceptives probably do not accelerate and may in fact inhibit progression of atherosclerosis in human beings.

We now suspect that in monkeys the antiatherogenic effect of oral contraceptives is accounted for by a direct effect of ethinyl estradiol, the potent estrogen in these formulations, on atherogenesis—an effect that is independent of any changes in plasma lipids. This hypothesis is further supported by findings we have recently made in our laboratory that treatment with estradiol of macaques fed atherogenic diets resulted in marked reduction in the uptake and metabolism of atherogenic low-density lipoproteins by the artery wall

and may thereby directly retard atherogenesis.[11] Although we have not yet studied effects of ethinyl estradiol on this process, it seems reasonable to speculate that it will be even more potent as an inhibitor of low-density lipoprotein uptake. Regardless of the mechanism, we conclude from our findings that contraceptive progestin-induced lowering of HDL is not atherogenic if a sufficiently potent estrogen is coadministered. The striking difference between the expected and observed outcome suggests a beneficial effect of ethinyl estradiol on the coronary wall.

REFERENCES

1. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham Study. Ann Intern Med 1979;90:85-91.
2. Tikkanen MJ, Nikkila EA. Oral contraceptives and lipoprotein metabolism. J Reprod Med 1986;31:898-905.
3. Koritnik DR, Clarkson TB, Adams MR. Cynomolgus macaques as models for evaluating effects of contraceptive steroids on plasma lipoprotein and coronary artery atherosclerosis. In: Blye R, Gregoire A, eds. Contraceptive steroids: pharmacology and safety. New York: Plenum Press, 1986:303-19.
4. Hamm TE Jr, Kaplan JR, Clarkson TB, Bullock BC. Effects of gender and social behavior on the development of coronary artery atherosclerosis in cynomolgus macaques. Atherosclerosis 1983;48:221-33.
5. Adams MR, Kaplan JR, Clarkson TB, et al. Ovariectomy, social status, and atherosclerosis in cynomolgus monkeys. Arteriosclerosis 1985;5:192-200.
6. Adams MR, Kaplan JR, Manuck SB, Koritnik DR, Parks JS, Wolfe MS, Clarkson TB. Inhibition of coronary artery atherosclerosis by 17-beta estradiol in ovarectomized monkeys: lack of an effect of adding progesterone. Arteriosclerosis (in press).
7. Adams MR, Clarkson TB, Koritnik DR, Nash HA. Contraceptive steroids and coronary artery atherosclerosis in cynomolgus macaques. Fertil Steril 1987;47:1010-8.
8. Clarkson TB, Shively CA, Morgan TM, Koritnik DR, Adams MR, Kaplan JR. Oral contraceptives and coronary artery atherosclerosis of cynomolgus monkeys. Obstet Gynecol 1990;75:217-22.
9. Mishell DR Jr. Use of oral contraceptives in women of older reproductive age. Am J Obstet Gynecol 1988; 158:1652-7.
10. Stampfer MJ, Willett WC, Colditz GA, Speizer FE, Hennekens CH. A prospective study of past use of oral contraceptive agents and risk of cardiovascular diseases. N Engl J Med 1988;319:1313-7.
11. Wagner JD, Clarkson TB, St. Clair RW, Schwenke DC, Adams MR. Estrogen replacement therapy and coronary artery atherogenesis in surgically postmenopausal monkeys [Abstract]. Circulation 1989;80:II-331.

Oral contraceptives and myocardial infarction

Ian H. Thorneycroft, PhD, MD
New Orleans, Louisiana

The risk of myocardial infarction in contraceptive users is limited to women over 35 years of age who smoke. The cause of myocardial infarction in oral contraceptive users is thrombotic and not atherosclerotic. Minor lipid changes have no clinical relevance to myocardial infarction in contraceptive pill users and do not appear to increase coronary plaques. (Am J Obstet Gynecol 1990;163:1393-7.)

Key words: Myocardial infarction, oral contraceptives

A number of both case-control and prospective studies have examined the relationship between oral contraceptives and myocardial infarction. A thorough review of this epidemiologic evidence is not the purpose of this article.[1] The most recent analyses of the Royal College of General Practitioners' Study are reviewed in detail, as are potential mechanisms by which oral contraceptives could cause myocardial infarction. Particular attention is paid to the relevance of lipid studies.

From the Department of Obstetrics and Gynecology, Tulane University.
Reprint requests: Ian H. Thorneycroft, PhD, MD, Department of Obstetrics and Gynecology, University of South Alabama, 2451 Fillingim St., Mobile, AL 36617.
6/0/22640

Epidemiology

Early retrospective studies demonstrated approximately a fourfold increase in myocardial infarction in women ingesting oral contraceptives. More recent studies and several prospective studies have not come to the same conclusion.[1] The 1981 analysis of the prospective Royal College of General Practitioners' Study[2] found an increased risk for myocardial infarction in women ingesting oral contraceptives only if they were over the age of 35 years and smoked. The Oxford Family Planning Study[3] found no association with current or past use of oral contraceptives in the analysis of their data published in 1987. They also noted that smokers were at increased risk. The Puget Sound and the Kaiser Walnut Creek studies in the United States failed to confirm an associated risk.[4, 5]

Table I. Royal College of General Practitioners' data: Factors increasing relative risk of myocardial infarction

	Relative risk	*95% confidence interval*
Oral contraceptive use		
Never	1.0	
Previous	1.0	0.7-1.6
Current	1.8	0.9-3.6
Smoking		
Nonsmoker	1.0	
<15 cigarettes/day	1.7	1.1-2.7
>15 cigarettes/day	4.3	2.6-6.9
Toxemia	2.8	1.7-4.8
Hypertension	2.4	1.4-4.1
Diabetes	6.9	1.1-43.8

Data from Croft P, Hannaford PC. Br Med J 1989;298: 165-8.

Table II. Risk factors for myocardial infarction and use of oral contraceptives

	Oral contraceptive use		
Risk factor	*Never*	*Former*	*Current*
Hypertension			
No	1.0	1.5	2.0*
Yes	5.4*	2.6*	7.7*
Toxemia			
No	1.0	1.3	2.0
Yes	2.6*	4.0*	3.2*
Cigarettes			
Never	1.0	1.1	0.9
<15/day	2.0	1.3	3.5*
>15/day	3.3*	4.3*	20.8*

Data from Croft P, Hannaford PC. Br Med J 1989;298: 165-8.

*Significant rise.

The most recent analysis of the data of the Royal College of General Practitioners' Study by Croft and Hannaford[6] identified several factors that increase the relative risk of myocardial infarction in young women (Table I). Neither current nor past oral contraceptive users had an increased risk of myocardial infarction. Although current users had a 1.8 times relative risk, the 95% confidence interval includes 1.0. Smoking was a significant risk factor for myocardial infarction. Smoking <15 cigarettes a day led to a 1.7-fold increased risk and >15 cigarettes a day led to a 4.3-fold increased risk. A history of toxemia, hypertension, or diabetes was also a significant risk factor for myocardial infarction.

The data were also analyzed to determine if oral contraceptive use further increased the other known risks of myocardial infarction (Table II). Hypertension and toxemia were again associated with myocardial infarction, but there were no differences among never, former, and current oral contraceptive users. The confidence intervals were wide. More data are needed on any potential interaction between oral contraceptives and hypertension, toxemia, diabetes, and abnormal baseline lipid studies. Current use of oral contraceptives, in combination with smoking >15 cigarettes a day, had the highest high relative risk for myocardial infarction. Oral contraceptive use amplified the risk associated with smoking. These data expand those previously published by the Royal College of General Practitioners, which demonstrated that the real risk factor for myocardial infarction in women using oral contraceptives is smoking. Pills of <50 μg have not been associated with any myocardial infarctions.[3] The differences between early studies and recent analyses are that the early studies did not control for smoking and patients smoking <15 cigarettes a day were classified as nonsmokers.

Table III. Relative risk of major coronary disease among past users of oral contraceptives based on time since last use

Time since last use (yr)	*No. of cases*	*Relative risk*	*95% confidence interval*
Never used	339	1.0	—
<1	1	0.3	0.1-2.2
1-3	8	0.7	0.3-1.5
3-5	8	0.6	0.3-1.2
5-10	33	0.7	0.5-1.0
10-15	54	1.2	0.9-1.6
>15	18	1.0	0.7-1.4

Data from Stampfer MJ, Willett WC, Colditz GA, Speizer FE, Hennekens CH. N Engl J Med 1988:319:1313-7. Reprinted by permission of the *New England Journal of Medicine.*

If the rate of myocardial infarction was as great as originally estimated (fourfold increase), then mortality studies would clearly show an increased rate of death from ischemic heart disease or myocardial infarction after the introduction of oral contraceptives. Fig. 1 illustrates such data from the United Kingdom.[7] After the introduction of oral contraceptives, cardiovascular disease declined slightly and ischemic heart disease was stable. Therefore no relationship could be found between the introduction of oral contraceptives and heart disease.

Causes of myocardial infarction

Possible causes of myocardial infarction in women ingesting oral contraceptives include increased atherogenesis, increased coronary thrombosis, and coronary artery spasm.

The belief that increased atherosclerosis may cause myocardial infarction is derived from the cholesterol hypothesis. Elevated total and low-density lipoprotein

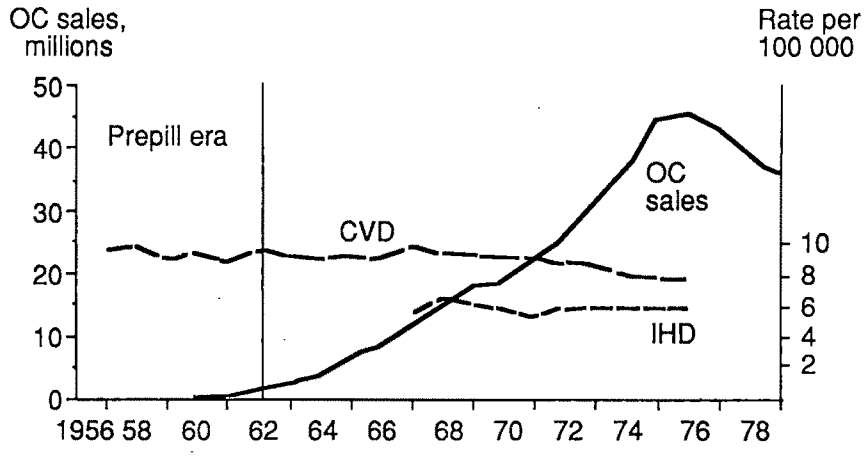


Fig. 1. Mortality rates in women, ages 15 to 44 years inclusive, per 100,000 population in England and Wales for cerebrovascular disease *(CVD)* and ischemic heart disease *(IHD)*. *OC*, Oral contraceptive. (From Wiseman RA, MacRae KD. Fertil Steril 1981;35:277-83. Reproduced with permission of the publisher, The American Fertility Society.)

Table IV. Relative risk of major coronary disease among past users of oral contraceptives based on duration of use

Duration of use (yr)	*No. of cases*	*Relative risk*	*95% confidence interval*
None	339	1.0	—
<1	45	1.0	0.7-1.4
1-3	32	1.0	0.7-1.4
3-5	20	1.0	0.6-1.7
5-10	25	0.8	0.5-1.2
<10	10	0.7	0.4-1.2

Data from Stampfer MJ, Willett WC, Colditz GA, Speizer FE, Hennekens CH. N Engl J Med 1988;319:1313-7. Reprinted by permission of the *New England Journal of Medicine.*

(LDL) cholesterol or decreased high-density lipoprotein (HDL) cholesterol are atherogenic and associated with an increased rate of myocardial infarction. Progestins present in oral contraceptives lower HDL and raise LDL cholesterol; all estrogens have the opposite effect. Older, high-dose progestin oral contraceptive preparations were associated with what could be interpreted as adverse changes in lipid profiles.[8] The lipid profile depends on the balance between the estrogen and progestin components. Modern low-dose oral contraceptives are not associated with clinically significant lipid changes.[9] If the mechanism of action of oral contraceptives in increasing myocardial infarction is related to lipid changes, then the following findings should be evident: (1) Past use of oral contraceptives should increase myocardial infarction. (2) The rate of myocardial infarction should increase with increased duration of use. (3) Oral contraceptives should be associated with adverse lipid profiles. (4) These adverse lipid changes should translate into increased coronary artery atherosclerosis. (5) Patients ingesting preparations with adverse lipid changes or a high progestin content should have a higher myocardial infarction rate. (6) Autopsy and angiographic studies should show premature and extensive coronary atherosclerosis.

Table III, which is adapted from the Nurses Health Study,[10] clearly demonstrates that women who used oral contraceptives in the past have no increased risks of myocardial infarction. Nine other studies have demonstrated that past use of oral contraceptives is not associated with an increased rate of myocardial infarction.[10] One study showed a small subset at increased risk[11]; however, Stampfer et al.[10] were not able to confirm that this subset was at greater risk. Table IV illustrates that duration of use also was not related to the incidence of myocardial infarction.

Lipid changes in women ingesting oral contraceptives have been the subject of many publications. Fig. 2 illustrates LDL and HDL levels from selected oral contraceptives from the study of Wahl et al.[8] The vast majority of values from women ingesting oral contraceptives are well within the normal range and unlikely to be atherogenic, even though their mean values may be changed.

Whether adverse lipid changes noted with oral contraceptive use translate into coronary atherosclerosis is

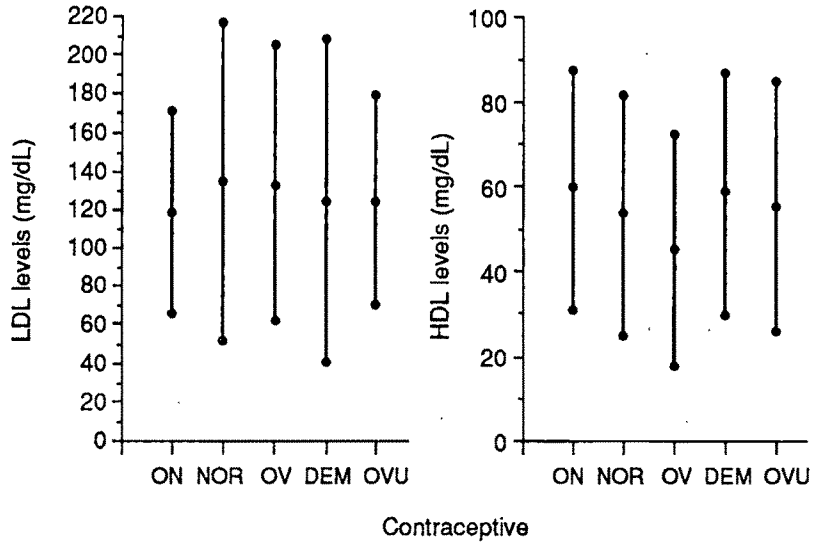


Fig. 2. LDL and HDL levels of selected oral contraceptives (mean ± 2 SD). *ON,* Ortho-Novum, norethindrone + mestranol; *NOR,* Norlestrin, norethindrone acetate + ethinyl estradiol; *OV,* Ovral, norgestrel + ethinyl estradiol; *DEM,* Demulen, ethynodiol diacetate + ethinyl estradiol; *OVU,* Ovulen, ethynodiol diacetate + mestranol. (Data from Wahl P, Walden C, Knopp R, et al. N Engl J Med 1983;30:862-7.)

a difficult question to answer. The elegant cynomolgus macaque studies are reviewed in Adams' presentation in this symposium.[12, 13] Briefly, significant lipid changes were induced in macaque monkeys given oral contraceptive preparations that lowered HDL and raised LDL cholesterol. These lipid changes induced by oral contraceptives did not translate into increased atherogenesis. In fact, the macaque monkeys ingesting oral contraceptives had fewer coronary plaques than did control animals. Such an experiment is not possible in women. However, Engel et al.[14] analyzed coronary angiograms in 76 young women who had suffered a myocardial infarction. Forty-two women used oral contraceptives and 34 did not. Thirty-six percent of women using oral contraceptives had diffuse atherosclerosis, whereas the 79% not using oral contraceptives had diffuse atherosclerosis. Clearly, women undergoing myocardial infarction while taking oral contraceptives did not have atherosclerotic myocardial infarction. Numerous autopsy studies have shown that myocardial infarction in women ingesting oral contraceptives was thrombotic and not atherosclerotic.[14]

Some lipid studies have demonstrated that high-dose levonorgestrel-containing oral contraceptives have the more unfavorable lipid profile. This is not true for all studies. Comparisons between progestins are difficult, because unequivalent doses of progestin and estrogen are usually compared. It is the dose of the progestin and not the specific progestin that affects lipid levels. Croft and Hannaford[15] were unable to find any evidence or examination of their data base to support the hypothesis that levonorgestrel increases the risk of myocardial infarction over other progestins. The relative risk was 0.55 for levonorgestrel and 1.24 for other progestins. The confidence intervals both included 1.0. These differences were not significant. One study showed a dose-response curve between myocardial infarction and the dose of progestin.[16] However, patients did not take the various progestin doses in the same time span. Those patients who ingested the higher doses were probably older, since these were the older preparations. The increased rate of myocardial infarction may simply be the result of the patient's age.

Comment

Myocardial infarctions are extremely rare events in women ingesting oral contraceptives and are usually limited to women over the age of 35 years who smoke. Women who are hypertensive, diabetic, or have a history of toxemia have a higher rate of myocardial infarction. Women with hypertension and toxemia are no more likely to have myocardial infarction if they are ingesting oral contraceptives; however, the confidence limits are large. The risk for persons with diabetes ingesting oral contraceptives is unknown.

The mechanism of action for myocardial infarction in women ingesting oral contraceptives is not atherosclerotic but thrombotic or possibly spasm. Accelerated atherosclerosis is not a characteristic feature in patients with myocardial infarction who use oral contraceptives. Minor lipid changes, noted mainly with oral contraceptives with a high progestin content, may be statistically significant but without clinical significance. It is too simplistic and invalid to extrapolate to oral contra-

ceptive data from epidemiologic studies examining the role of cholesterol in myocardial infarction in men. As Adams et al. demonstrated in their contribution to this symposium, the estrogen component in birth control pills may directly protect the coronary arteries from such atherosclerosis by inhibiting the entry of LDL cholesterol into blood vessel walls.

REFERENCES

1. Realini JP, Goldzieher JW. Oral contraceptives and cardiovascular disease: a critique of the epidemiologic studies. Am J Obstet Gynecol 1985;152:729-98.
2. Royal College of General Practitioners' oral contraceptive study. Further analysis of mortality in oral contraceptive users. Lancet 1981;1:541.
3. Mant D, Villard-Mackintosh L, Vessey MP, Yeates D. Myocardial infarction and angina pectoris in young women. J Epidemiol Commun Health 1987;41:215-9.
4. Petitti DB, Wingerd J, Pellegrin F, Ramcharan S. Risk of vascular disease in women: smoking, oral contraceptives, noncontraceptive estrogens and other factors. JAMA 1979;242:1150-4.
5. Porter JB, Jick H, Walker AM. Mortality among oral contraceptive users. Obstet Gynecol 1987;70:29-32.
6. Croft P, Hannaford PC. Risk factors for acute myocardial infarction in women: evidence from the Royal College of General Practitioners' oral contraception study. Br Med J 1989;298:165-8.
7. Wiseman RA, MacRae KD. Oral contraceptives and the decline in mortality from circulatory disease. Fertil Steril 1981;35:277-83.
8. Wahl P, Walden C, Knopp R, et al. Effect of estrogen/progestin potency on lipid/lipoprotein cholesterol. N Engl J Med 1983;308:862-7.
9. Notelovitz M, Feldman EB, Gillespy M, Gudat J. Lipid and lipoprotein changes in women taking low-dose, triphasic oral contraceptives: a controlled, comparative, 12-month clinical trial. Am J Obstet Gynecol 1989;160:1269-80.
10. Stampfer MJ, Willett WC, Colditz GA, Speizer FE, Hennekens CH. A prospective study of past use of oral contraceptive agents and risk of cardiovascular disease. N Engl J Med 1988;319:1313-7.
11. Slone D, Shapiro S, Kaufman DW, Rosenberg L, Miettinen OS, Stolley PD. Risk of myocardial infarction in relation to current and discontinued use of oral contraceptives. N Engl J Med 1981;305:420-4.
12. Adams MR, Clarkson TB, Koritnik DR, Nash HA. Contraceptive steroids and coronary artery atherosclerosis in cynomolgus macaques. Fertil Steril 1987;47:1010-8.
13. Clarkson TB, Adams MR, Kaplan JR, Shively CA, Koritnik DR. From menarche to menopause: coronary artery atherosclerosis and protection in cynomolgus monkeys. Am J Obstet Gynecol 1989;160:1280-5.
14. Engel H-J, Engel E, Lichtlen PR. Coronary atherosclerosis and myocardial infarction in young women—role of oral contraceptives. Eur Heart J 1983;4:1-8.
15. Croft P, Hannaford P. Risk factors for acute myocardial infarction in women [Letter]. Br Med J 1989;298:674.
16. Meade TW, Greenberg G, Thompson SG. Progestogens and cardiovascular reactions associated with oral contraceptives and a comparison of the safety of 50- and 30 μg oestrogen preparations. Br Med J 1980;280:1157-61.

PART II: A NEW ERA WITH GESTODENE

Clinical acceptability of monophasic gestodene

Bernd Düsterberg and Klaus Brill
Berlin, West Germany

The monophasic low-dose contraceptive containing 30 μg of ethinyl estradiol and 75 μg of gestodene has been tested in numerous clinical studies to determine contraceptive reliability, cycle control, and influence on metabolism and to uncover concomitant symptoms. Clinical data have been derived from continuous phase III studies. Now that the preparation has been introduced in several countries, the opportunity to assemble comprehensive experience has been taken by initiating phase IV studies. Documentation on 600,000 therapy cycles with monophasic gestodene among 100,000 women is already available. The drop-out rate was low. A large number of women participated in the clinical studies for a long time—some up to 3 years. Study results showed that contraceptive reliability was high and cycle control was excellent. The gestodene combination was well tolerated. The frequency of concomitant symptoms was low. The monophasic gestodene meets the basic criteria essential for an oral contraceptive. (Am J Obstet Gynecol, 1990;163:1398-1404.)

Key words: Combination oral contraceptive, contraceptive reliability, cycle control, gestodene

In 1987 a new low-dose combination oral contraceptive agent containing 75 μg of gestodene and 30 μg of ethinyl estradiol (Fig. 1) received its first marketing approval from the German authorities.[1] The preparation is based on the novel progestogen gestodene. Certain advantages are to be anticipated from the clinical application considering the endocrine pharmacologic profile of the compound.

The potent progestational activity of gestodene[2] should provide contraceptive reliability even at extremely low-dose levels. Antiestrogenic properties of the compound can reduce undesirable estrogenic effects in the endometrium and in metabolism.[2] Contraceptive doses of gestodene are virtually without any androgenic activity[2]; thus the occurrence of acne, large increases in body weight, and negative influences on lipid metabolism is unlikely. Finally, the slight antialdosterone effect of gestodene[3] may have some advantages for blood pressure during long-term application. Consequently, the pharmacologic profile of the new progestogen fulfilled all prerequisites for the development of an oral contraceptive based on this progestogen.

The aims of the clinical studies in phases III and IV were above all to examine contraceptive reliability, cycle control, and frequency of concomitant symptoms resulting from treatment with monophasic gestodene. These factors, in addition to the well-known beneficial effects of oral contraceptives, may have an influence on the acceptability of the compound (Fig. 2). In this context, compliance must be considered. It can be assessed to some extent by the drop-out rate in clinical trials.

From the Research Laboratories, Schering AG.
Reprint requests: Bernd Düsterberg, PhD, Research Laboratories, Schering AG, Postfach 65 03 11, D-1000 Berlin 65, West Germany.
6/0/22641

Material and methods

Subjects and design of trials. Healthy, sexually active female volunteers, ideally <35 years of age, were asked to take the preparation for periods varying between 6 and 36 cycles in the different studies. Each volunteer provided informed consent and was considered to have sufficient intelligence and motivation to follow instructions and continue the studies for the anticipated periods of observation. They were screened for contraindications to the use of oral contraceptives. A complete history and physical examination were performed before they entered the trial. Follow-up evaluations were performed regularly throughout the trial periods according to the trial protocols.

Sizes of clinical trials. The sizes of seven multicenter phase III studies are shown in Table I.[4-9] The trials were conducted mainly in European and in some Latin American countries according to local institutional regulations. The number of women recruited and consequently the number of cycles covered varied considerably. In total 6854 women participated in 69,978 cycles of treatment with monophasic gestodene in phase III studies.

In addition, data have been obtained on 523,477 cycles in 95,906 women participating in a phase IV study.[10] This trial was performed in Germany according to German regulations. This means that for this type of trial, the most relevant conditions—for instance, the

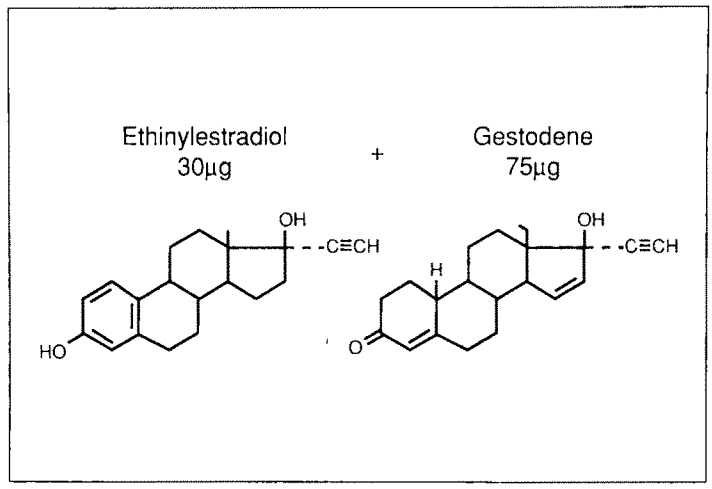


Fig. 1. Structural formula of ethinyl estradiol and gestodene.

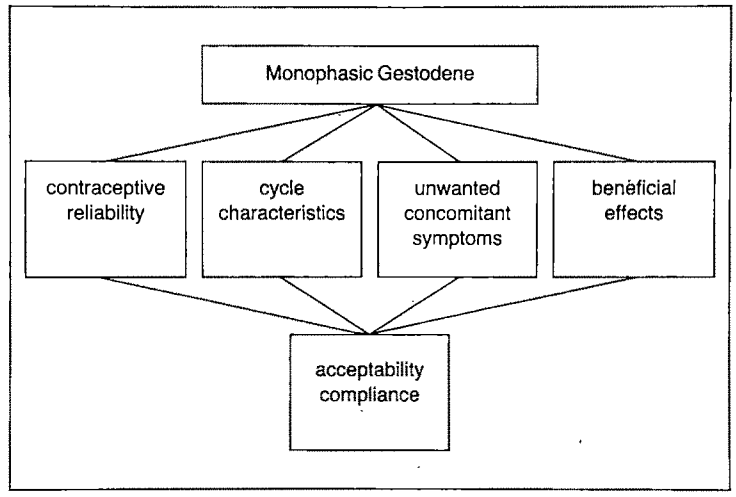


Fig. 2. Factors influencing patient acceptability of monophasic gestodene.

Table I. Clinical trials with monophasic gestodene

Study	*Country*	*No. of women*	*No. of cycles*	*Duration (cycles)*
Phase III				
A	West Germany	3,267	36,771	18
B	Europe, Latin America	1,095	14,281	24
C	Europe, Latin America	905	7,268	20
D	Europe, Latin America	817	4,574	12
E	United Kingdom	414	4,704	36
F	Switzerland	184	1,381	12
G	Italy	172	999	6
SUBTOTAL		6,854	69,978	
Phase IV				
H	West Germany	95,906	523,477	6
TOTAL		102,760	593,455	

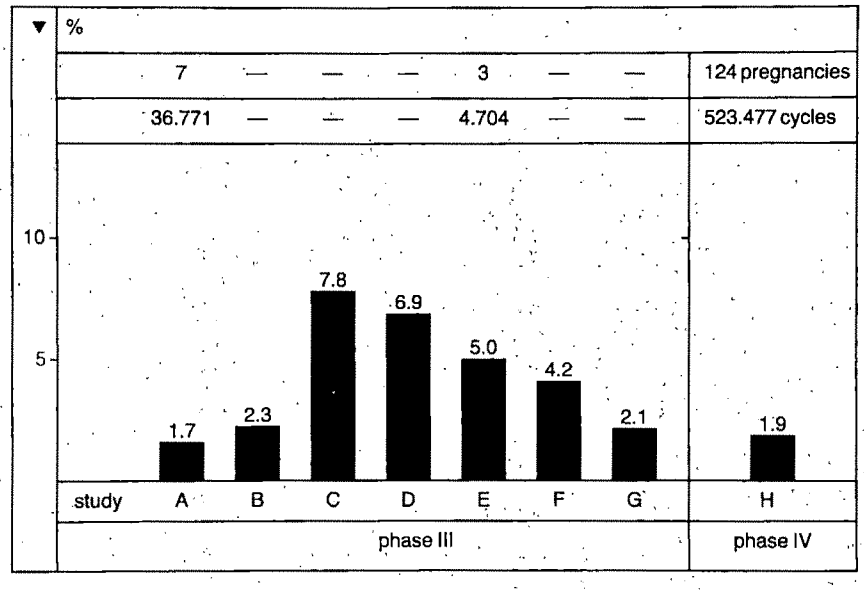


Fig. 3. Percentage of cycles with "missed pills" and number of pregnancies.

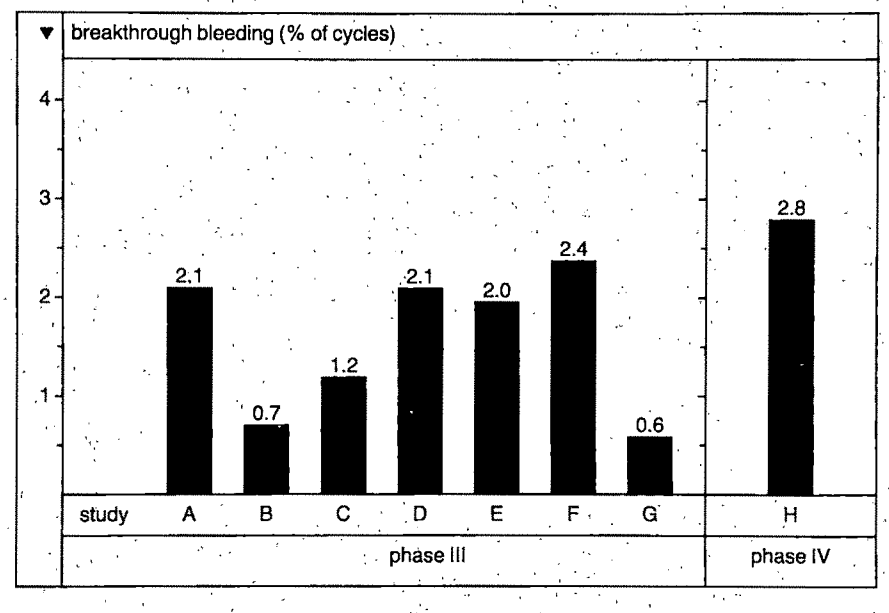


Fig. 4. Breakthrough bleeding rate during treatment with monophasic gestodene.

Table II. The contraceptive efficacy of monophasic gestodene

No. of women	3,267
Cycles of use	36,771
Period of observation:	18 cycles maximum
Pearl index	
Tablet failure	0.07
User failure	0.16
Range for low-dose oral contraceptives	0.03-0.22*

*See Taubert HD, Kuhl H. Kontrazeption mit Hormonen. Stuttgart: George Thieme Verlag, 1981.

trial protocol, the record forms, and the performance of the trial in general—are comparable with those of the phase III studies.

Results

Contraceptive reliability. Few pregnancies were recorded in any of the phase III studies and the phase IV trial (Fig. 3). Despite incidences of one to eight or more missed tablets in many of the cycles in the various studies, pregnancies occurred in only three out of eight trials. Most of these pregnancies were attributable to

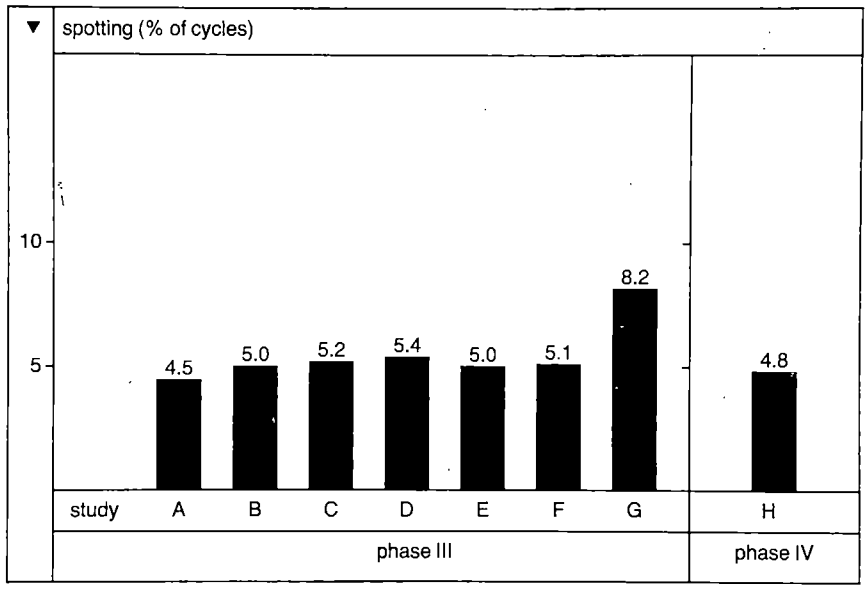


Fig. 5. Spotting rate during treatment with monophasic gestodene.

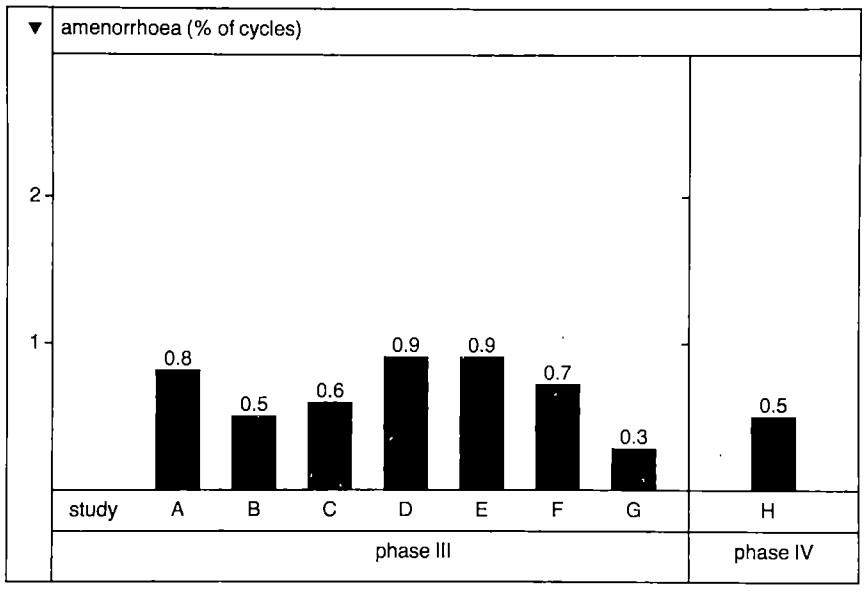


Fig. 6. Amenorrhea rate during treatment with monophasic gestodene.

errors in tablet taking. Some pregnancies were presumed to be from method failures.

Based on the most comprehensive phase III trial covering 36,721 cycles in 3267 women and the maximum observation period of 18 months, the Pearl index (as a measure of contraceptive reliability) was 0.07 for tablet failures and 0.16 for user failures (Table II).[4] Thus the uncorrected Pearl index is about 0.2, a value confirmed by the phase IV trial. Although monophasic gestodene has an extremely low steroid content, its efficacy is as good or better than that of most currently available low-dose preparations.[9]

Cycle control. Bleeding irregularities were recorded as "breakthrough bleeding" when the quantity required sanitary protection and as "spotting" when the amount was tolerable without such protection. The frequency of intracyclic bleeding was very low during treatment with monophasic gestodene. Breakthrough bleeding was reported in 0.6% to 2.8% of the cycles documented in the trials (Fig. 4). Spotting occurred for an incidence of 4.5% to 8.2%, mainly during the first cycles of use (Fig. 5). Amenorrhea, which was defined as an absence of bleeding during the tablet-free interval, was recorded in <1% of the cycles (Fig. 6).

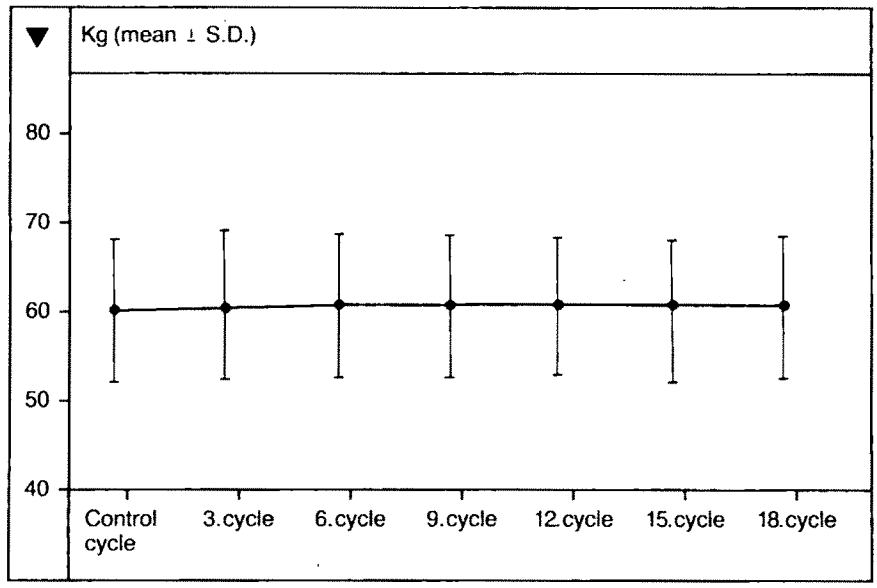


Fig. 7. Body weight changes during treatment with monophasic gestodene (phase III trial, $n = 3267$).

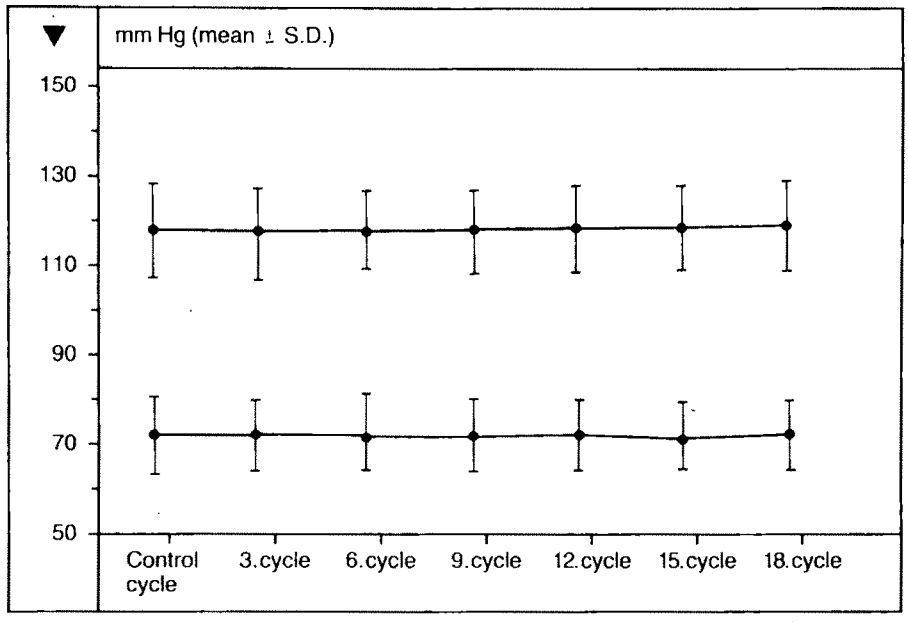


Fig. 8. Blood pressure changes during treatment with monophasic gestodene (phase III trial, $n = 3267$). (From Brill K, Schnitker J, Albring M. Langzeiterfahrungen mit dem niedrigdosierten Ovulationshemmer Femovan. Gyne 1989;2:47-50.)

Incidence of concomitant symptoms. Overall, the incidence of concomitant symptoms reported by the women during the trials was very low. As shown in Table III, only breast tension, headache, nausea, nervousness, dizziness, and depression were recorded in >1% of the women after six cycles of monophasic gestodene use. Nausea and vomiting occurred fairly frequently in the initial cycles of use and dropped thereafter. The frequency of breast tension and headaches remained around the pretreatment level. Migraine-type headaches and varicose disorders were practically uninfluenced.

Acne and edema were recorded as objectifiable symptoms in a few cases (Table III). Regarding the number of women still participating in the study at the respective times of assessment, there was a continuous decline in the rate of acne. The number of cases of edema dropped continuously with the progressing trials.

Body weight and blood pressure. Monophasic gestodene had essentially no pronounced effects on body weight in most women. As a representative example of the results of all trials, the findings of the largest phase III study are presented.[4] The mean values of body weight changed only by a maximum of 0.2 kg in the

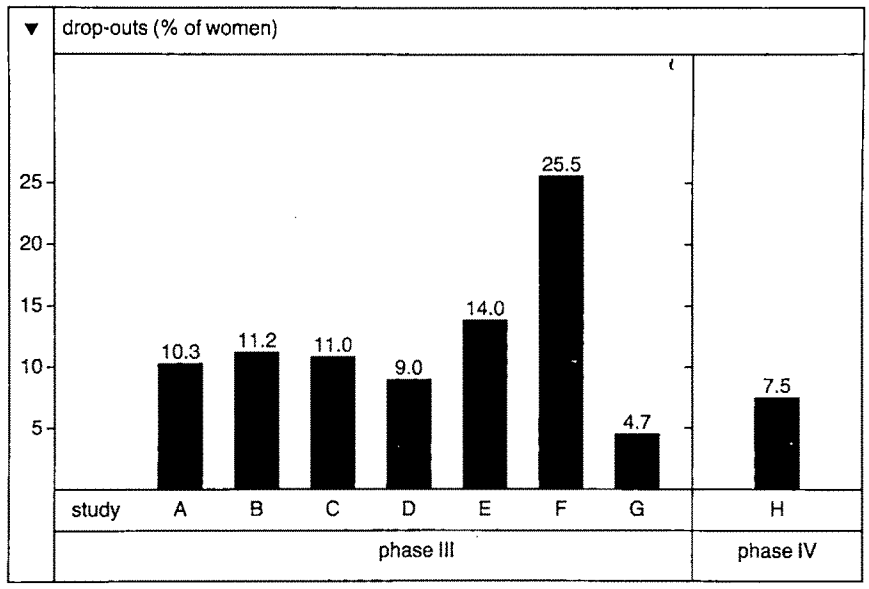


Fig. 9. Drop-out rate of clinical trials with monophasic gestodene.

Table III. Concomitant symptoms after six cycles of treatment with monophasic gestodene: percentage of assessed women

	Concomitant symptoms							
Study	*Breast tension*	*Headache*	*Nausea*	*Nervousness*	*Dizziness*	*Depression*	*Acne*	*Edema*
Phase III								
A	9.3	12.3	2.5	8.4	2.9	3.9	5.8	0.8
B	5.4	5.3	1.6	6.4	1.7	1.5	2.8	0.5
C	<1.0	1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
D	7.0	11.0	6.0	2.0	1.0	1.0	1.0	1.0
E	4.0	3.0	1.0	1.0	1.0	2.0	2.0	0
F	2.5	13.1	1.5	0	0	0.5	2.5	0
G	12.4	4.4	0	0	0	0	3.1	0
Phase IV								
H	0.5	0.6	0.1	0	0		0.3	0

study population. No significant weight gain could be attributed to the oral contraceptive (Fig. 7).

Similarly, blood pressure remained stable and within normal clinical limits in the eight trials. In all but a very few instances, pretreatment levels were measured throughout the studies. At no time was there an indication that monophasic gestodene had any effect on systolic or diastolic blood pressure. The data of the different trials are consistent; the results of the phase III study illustrate the findings (Fig. 8).

Discontinuation rates. Drop-out rates for medical reasons are shown in Fig. 9. Of the women enrolled in the trials, between 4.7% and 25.5% discontinued the monophasic gestodene for different medical reasons. Usually the drop-out rate was about 10% or even less than 10%. Study F had been performed in a family planning clinic in very young women. Probably the different contraceptive behavior of these young women contributed to the drop-out rate.

Generally the most frequently reported complaints were spotting and breakthrough bleeding, which were seen in a maximum of 5% of participants. Weight gain and headaches led to a drop-out rate of 2% to 4%. Less than 2% of the women reported nausea or breast tension. Other events were very rare; the rate for all of these was <1% of the women enrolled.

Comment. Cycle control of monophasic gestodene was excellent. The incidence of concomitant symptoms commonly associated with the use of oral contraceptives was minimal. Mean body weight and blood pressure showed virtually no changes.

The participants' acceptance of the new preparation was remarkably high. Only about 10% of the women discontinued the monophasic gestodene for medical reasons. It can be concluded from data obtained in about 100,000 women and 600,000 cycles of use that monophasic gestodene is an effective and well-tolerated oral contraceptive.

REFERENCES

1. Hofmeister H, Wiechert R, Annen K, Laurent H, Steinbeck H. Δ^{15}-17α-Äthinylsteroide der Östranreihe. Verfahren zu ihrer Herstellung sowie diese enthaltende pharmazeutische Präparate. DE 25 46 062. Berlin/Bergkamen: Schering AG, 1975.
2. Elger W, Steinbeck H, Schillinger E, Losert W, Beier S. Endocrine-pharmacological profile of gestodene. Advances in Contraceptives Delivery Systems 1986;2:182-97.
3. Losert W, Casals-Stenzel J, Buse M. Progestogens with antimineralocorticoid activity. Drug Res 1985;35:459-71.
4. Brill K, Schnitker J, Albring M. Langzeiterfahrungen mit dem niedrigdosierten Ovulationshemmer Femovan. Gyne 1989;2:47-50.
5. Hoppe G. Gestoden, an innovative progestogen. Contraception 1988;37:493-501.
6. Wyeth research data on file.
7. Kirkman RJ. Interim results of a UK study comparing a new monophasic pill containing gestodene to Microgynon 30 and proceeding to open assessment. In: Elstein M, ed. Gestodene, development of a new gestodene-containing low-dose oral contraceptive. London: Parthenon Publishing.
8. Schering research data on file.
9. Taubert HD, Kuhl H. Kontrazeption mit Hormonen. Stuttgart: Georg Thieme Verlag, 1981.
10. Brill B. Abstract 477, XIII World Congress on Fertility and Sterility, Marrakesh, Morocco, October 1989.

Inhibition of ovulation by low-dose monophasic contraceptive containing gestodene*

Karl Thomas, MD, and Leo Vankrieken, BSc

Brussels, Belgium

Ovulation inhibition by the monophasic oral contraceptive containing 75 μg of gestodene and 30 μg of ethinyl estradiol was evaluated in 25 healthy volunteers for five cycles: a pretreatment cycle, three treatment cycles, and a posttreatment cycle. Serum luteinizing and follicle-stimulating hormones, estradiol, and progesterone levels were measured on days 8 through 17; progesterone was measured once, around day 21. Pelvic ultrasound examinations were performed on cycle days 6, 8, 10, 12, 14, and 16. In 18 subjects completing the study, luteinizing and follicle-stimulating hormone levels were strongly depressed in the treated cycles. From treated cycle days 8 to 17, luteinizing and follicle-stimulating hormone levels reached values on the lower limit of detection. Luteal activity was not detected in treated cycles. Follicular activity, which was reflected by estradiol levels, was most strongly depressed during the first treated cycle. Pelvic ultrasound examinations confirmed excellent inhibition of follicular maturation. Restoration of ovarian function in the posttreatment cycle was excellent and showed a midcycle hormonal profile identical to the pretrial cycle. (Am J Obstet Gynecol 1990;163:1404-10.)

Key words: Gestodene, oral contraception, ovulation inhibition

Gestodene is probably the most powerful synthetic gestogen currently used in humans. Consequently, it contributes to the development of very efficient hormonal progestogen-estrogen combinations for oral contraception. Nevertheless, the impact of recent hormonal contraceptive formulations containing gestodene on the hypophysial-ovarian axis,[1, 2] whose principal goal is the suppression of ovulation, needs more extensive documentation, because essentially only their metabolic effects have been carefully studied.[3] Therefore this study was undertaken of a low-dose monophasic contraceptive containing gestodene and ethinyl estradiol to evaluate the impact on the hypophysial-ovarian axis in a group of young ovulating women.

From the Physiology of Human Reproduction Research Unit, Department of Obstetrics and Gynecology, University of Louvain, Brussels, Belgium.
Reprint requests: Karl Thomas, MD, Physiology of Human Reproduction Research Unit, Department of Obstetrics and Gynecology, University of Louvain 5330, Avenue E. Mounier, 53, B-1200 Brussels, Belgium.
**This article was first published in the International Journal of Fertility 1989;34(suppl):10-21. Reprinted by the permission of the U.S. International Foundation for Studies in Reproduction, Inc.*
6/0/22639

Material and methods

Twenty-five healthy volunteers, ages 27 ± 0.9 years (mean ± SEM), with a mean cycle length of 28.2 ± 0.6 days (mean ± SEM), were selected for the study. Each subject was followed during eight consecutive cycles: a pretreatment control cycle when no medication was given, six cycles when medication was given, and a posttreatment recovery cycle. A contraceptive preparation containing 75 μg of gestodene with 30 μg of ethinyl

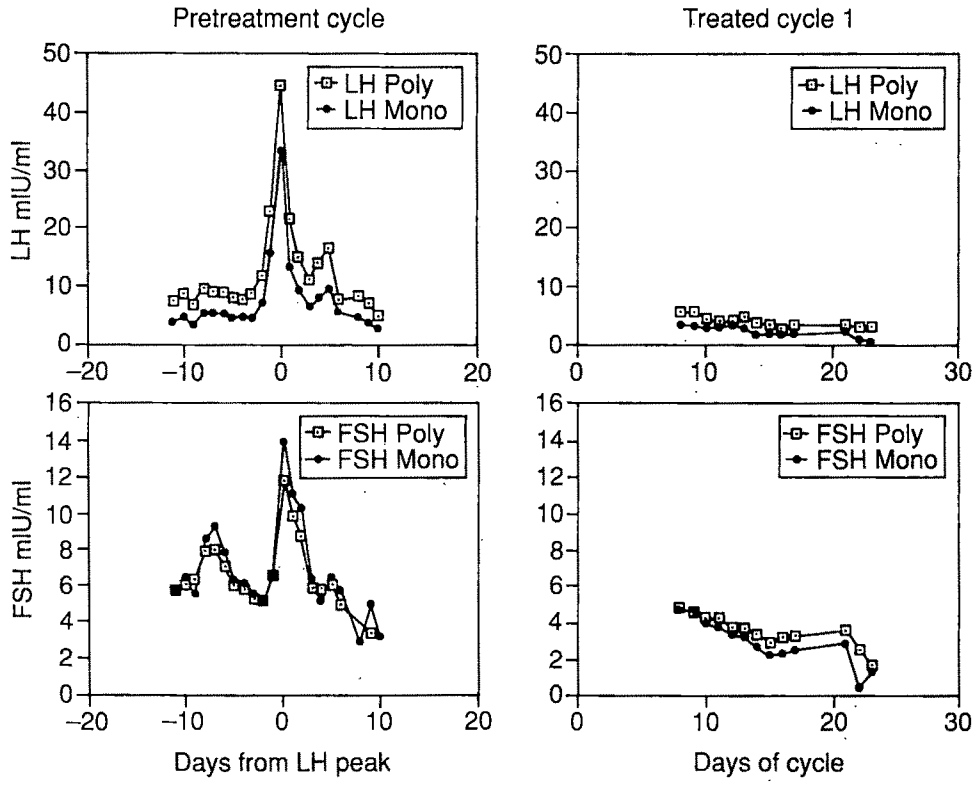


Fig. 1. Mean plasma LH and FSH concentrations measured by polyclonal and monoclonal RIA techniques in eight volunteers during pregnancy and first treatment cycles.

estradiol per daily dose was administered for 21 consecutive days during the treatment cycles, followed by a 7-day pill-free interval.

Plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and progesterone levels were assayed daily from cycle days 8 through 17 in the pretreatment cycle; treatment cycles 1, 3, and 6; and the posttreatment cycle. In addition, during the same cycles, progesterone was assayed once again at around cycle day 21, and pelvic ultrasound examinations were performed on days 6, 8, 10, 12, 14, and 16.

Of the 25 volunteers who began the study, 7 dropped out. Three were unable to come regularly for follow-up, two showed no signs of ovulation during the control cycle and therefore were excluded from the study, one dropped out after she became pregnant during the control cycle, and the seventh dropped out because of continuous spotting. All the results presented in this study are derived from the 18 volunteers who completed the entire study.

Blood plasma LH and FSH levels were assayed with polyclonal radioimmunoassay (RIA) systems (Amerlex, Amersham, England). Progesterone and 17 β-estradiol levels were assayed by classic RIA systems (Medgenix, Brussels, Belgium). In eight of the volunteers, plasma LH and FSH levels were simultaneously assayed by a monoclonal RIA system (Medgenix, Brussels, Belgium) during the pretreatment and first treatment cycles to compare the polyclonal and monoclonal RIA systems.

Ultrasound investigation of the ovaries was performed with a 3.5 MHz Diasonics DRF 100 sector scanner by means of the full bladder technique and the transabdominal approach. The diameters of the follicle structure were measured in both longitudinal and transverse planes from which the mean was calculated.

Results

The results obtained from the comparative study of the monoclonal and polyclonal RIA systems are shown in Fig. 1. In both the pretreatment and first treatment cycles, the fluctuations in mean LH levels obtained by the two systems are perfectly parallel to one another. As expected, the monoclonal values were somewhat lower but not statistically significantly different from those obtained with the polyclonal system. A similar situation is observed with the FSH assays, but the results are strictly superposable. This preliminary comparison validated the further use of only a polyclonal RIA system to establish our results.

Figs. 2 and 3 show the mean LH and FSH profiles of the 18 volunteers during the five explored cycles.

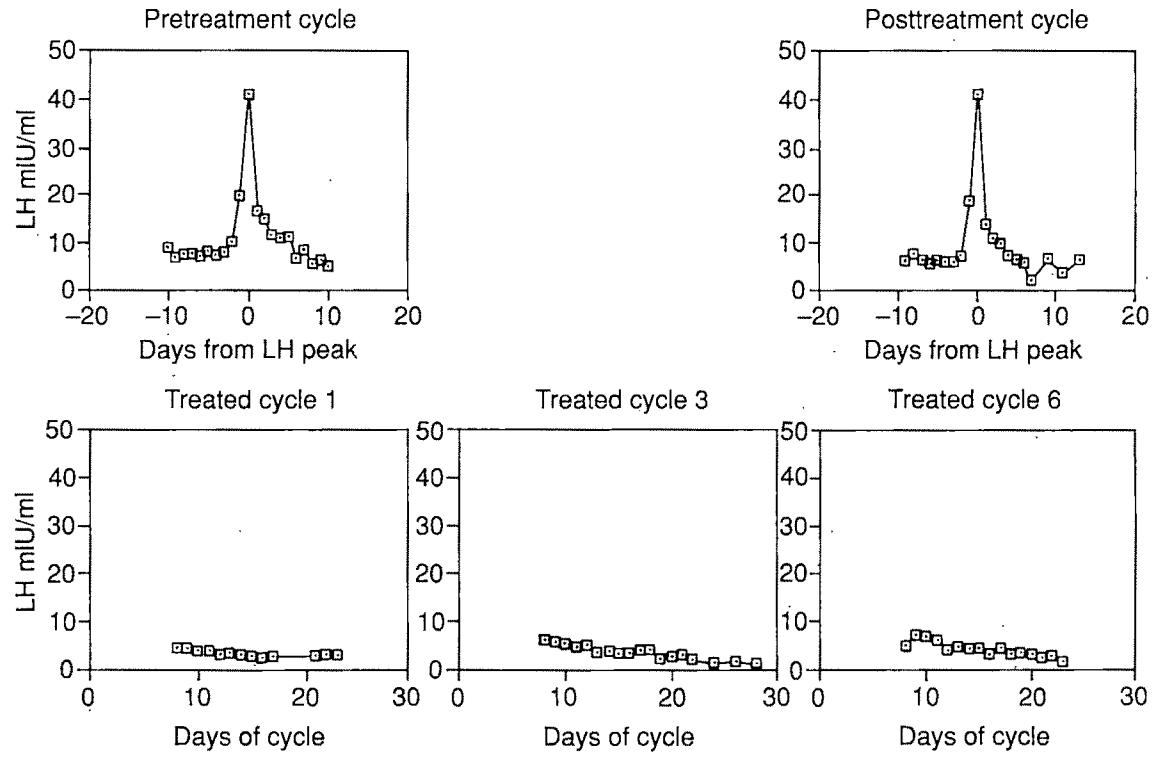


Fig. 2. Mean plasma LH levels in 18 volunteers during pre- and posttreatment cycles and during treatment cycles 1, 3, and 6 (75 μg of gestodene + 30 μg of ethinyl estradiol).

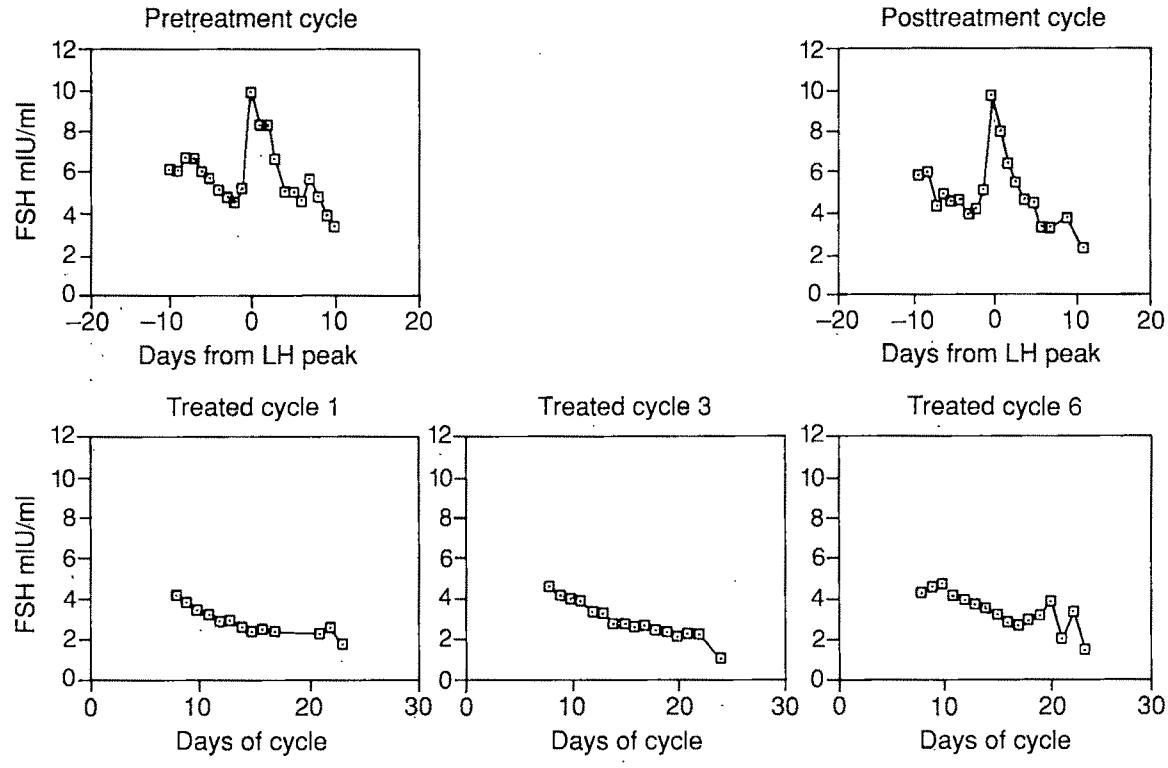


Fig. 3. Mean plasma FSH levels in 18 volunteers during pre- and posttreatment cycles and during treatment cycles 1, 3, and 6 (75 μg of gestodene + 30 μg of ethinyl estradiol).

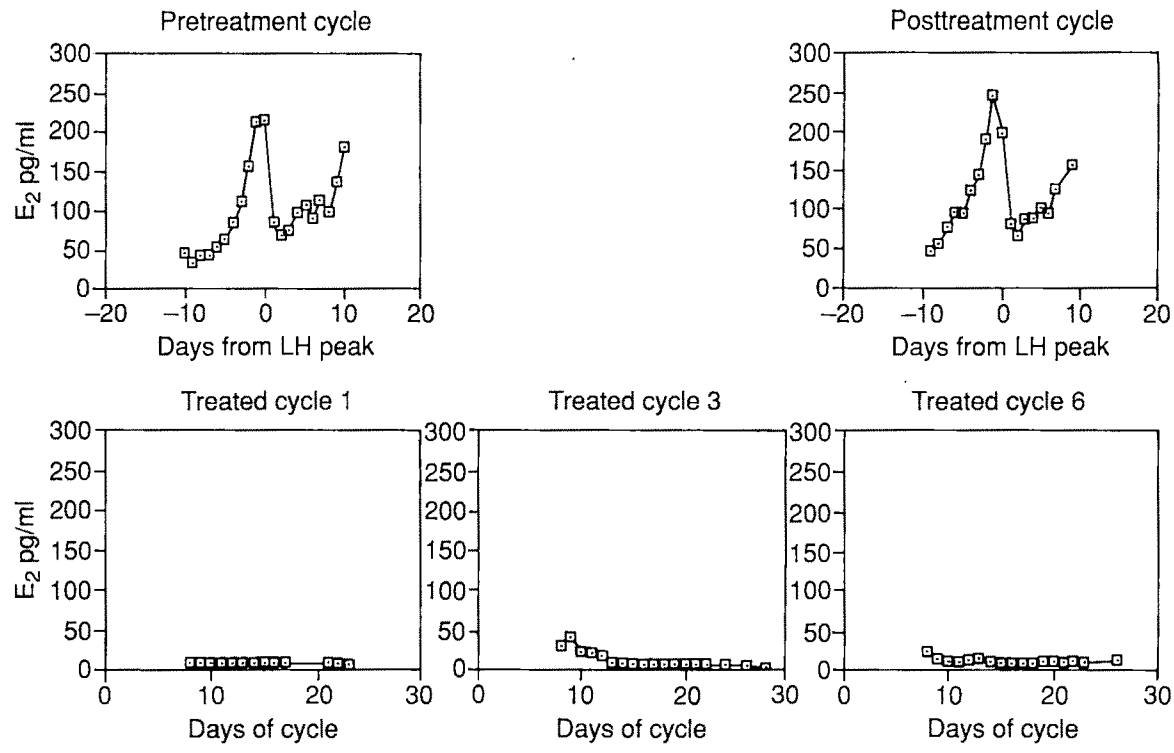


Fig. 4. Mean plasma 17-β estradiol levels in 18 volunteers during pre- and posttreatment cycles and during treatment cycles 1, 3, 6 (75 μg of gestodene + 30 μg of ethinyl estradiol).

For LH a complete suppression of the midcycle peak during the treatment cycles and its immediate reappearance in the posttreatment control cycle are observed. In the case of FSH secretion, the characteristic profile also disappears during the treatment cycle and again it reappears during the posttreatment control cycle.

A strong inhibition of estradiol secretion is already apparent in the first treatment cycle (Fig. 4). Again, a normal cyclic profile reappears as soon as treatment is stopped in the posttreatment cycle. Similar remarks may be made concerning the progesterone profiles (Fig. 5), in which total suppression of luteal activity was observed during treatment.

The hormone concentrations appear to have reached apparent stable suppressed values during the treatment cycles. If the hormonal concentrations during the treatment cycles are plotted on an expanded scale (Fig. 6), we can observe the impact of treatment not only on the characteristic hormonal fluctuations of the ovulatory cycle, as shown in Figs. 2 through 4, but also demonstrate that basal levels were significantly affected. In LH and FSH, a progressive significant decrease in concentration was clearly present in cycle 1 and to the same degree in treatment cycles 3 and 6.

However, the expanded scale for estrogens, shows a somewhat different pattern. During the first treatment cycle, nearly all serum estradiol concentrations are <25 pg/ml. However, in contrast, in the third and sixth treatment cycles, an elevated estradiol level was observed during the first few days of the hormonal investigation. For progesterone, the concentrations remain within stable limits during the treatment period (Fig. 5).

Fig. 7 shows the distribution of maximal 17β-estradiol (pg/ml) in each individual investigated cycle of the 18 volunteers. In the pre- and posttrial cycles, nearly all estradiol concentrations are in the mature follicular range values. In contrast, estradiol concentrations are strongly depressed (<25 pg/ml) in nearly all treatment cycles. However, in cycles 3 and 6, a few volunteers showed slight follicular activity and one even showed a high estradiol level.

The results of the ultrasound investigation of the ovaries are shown in Fig. 8. Here the data are expressed as the maximal follicular diameter, this from the leading follicle, measured in each investigated cycle. Again, the ovaries of pre- and posttreatment cycles show mature preovulatory follicles except in two cases in the posttreatment cycle, in which the follicular maturation was somewhat retarded. During all treatment cycles, follicular growth was blocked around 10 mm diameter

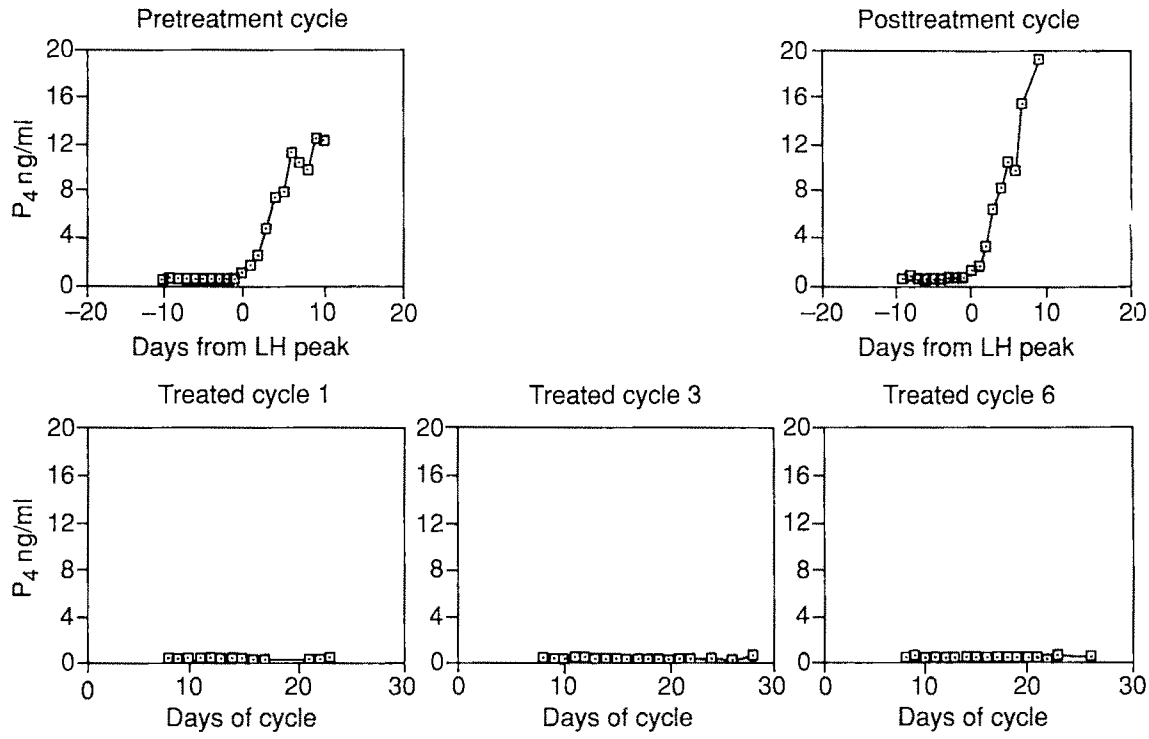


Fig. 5. Mean plasma progesterone estradiol levels in 18 volunteers during pre- and posttreatment cycles and during treatment cycles 1, 3, and 6 (75 μg of gestodene + 30 μg of ethinyl estradiol).

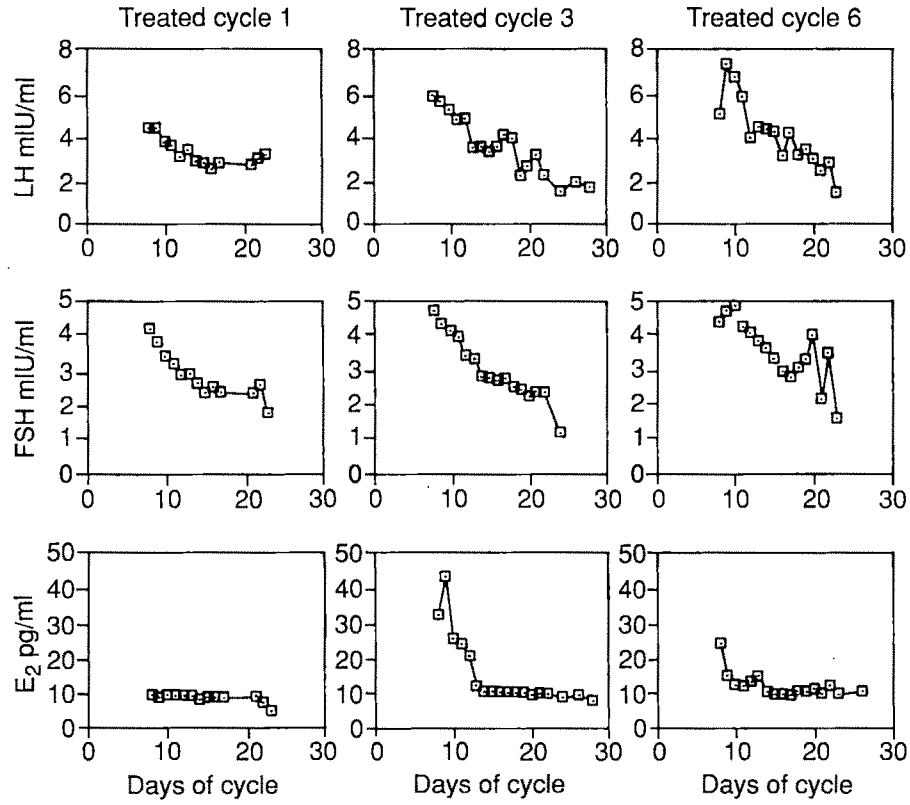


Fig. 6. Mean plasma LH, FSH, and 17β-estradiol concentrations in an expanded scale in the three explored cycles during treatment.

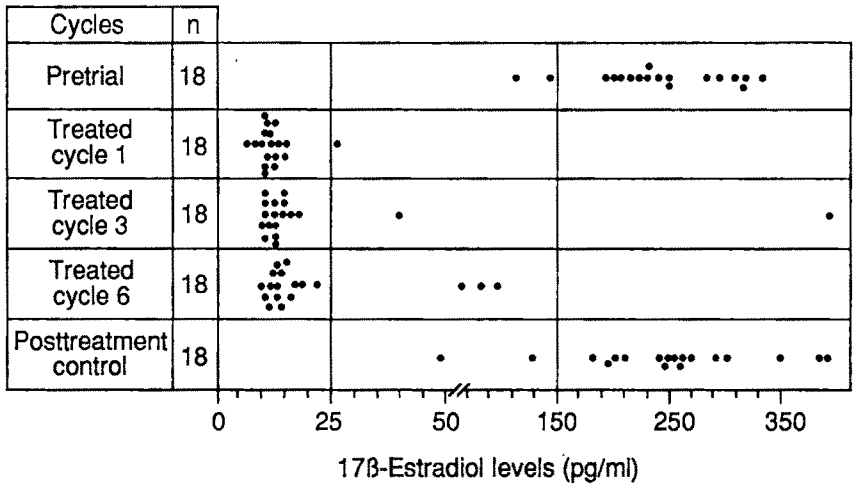


Fig. 7. Distribution of maximal 17β-estradiol levels in each investigated cycle of 18 volunteers.

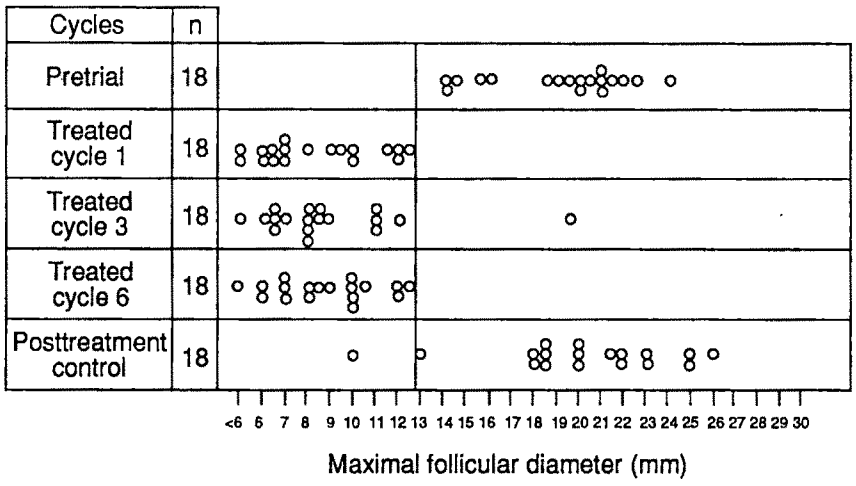


Fig. 8. Distribution of maximal follicular diameters in each investigated cycle of 18 volunteers. *Each open circle* represents the leading follicule of the cycle.

and only one in the third treatment cycle grew 12.5 mm. This exception is the same woman who had a high estradiol level during the third treatment cycle, as shown in Fig. 7. However, as with all the other volunteers, this woman did not develop any luteal activity during treatment.

Whereas oral contraceptives containing 75 μg of gestodene and 30 μg ethinyl estradiol have no clinically relevant influence on lipids, carbohydrates, and blood clotting,[3] from the foregoing evidence we can conclude that the impact on the hypophysial-ovarian axis results in a strong inhibition of this axis and consequently ovulation. The data of our study may be summarized as follows:

1. There is complete suppression of the midcycle LH surge and complete suppression of the characteristic fluctuations in FSH. Furthermore, from the first treatment cycle, basal levels of LH and FSH clearly show a progressive and significant decrease; this continues to be observed to the same degree in treatment cycles 3 and 6, thus demonstrating the efficacy of the formulation on basal gonadotropin secretion from the beginning of treatment.
2. Excellent blockage of follicular maturation is observed by ultrasonic assessment of the ovaries. This is further confirmed by very low plasma estradiol levels. However, the expanded scale of estrogen levels in the third and sixth treatment cycles shows slightly elevated estradiol levels in the first days of the hormonal investigation. This suggests that in a few volunteers follicular maturation may have already been initiated during the 7-day pill-free interval. This is not observed in the first treatment cycle when the volunteers begin treatment on the first day of menstruation. The pos-

sibility that follicular activity was initiated in some women, as observed in a few of our volunteers, during the third and sixth treatment cycles, raised the problem of the ideal length of the pill-free interval. The 7-day pill-free interval should be considered the maximum permitted. This problem is all the more acute because of the forgetfulness of some users. On the other hand, it has been shown that a 2-day interruption in the intake of this formulation failed to produce any marked increase in ovarian activity.[4]

3. The strong inhibition of ovarian secretion is also reflected by total suppression of all luteal activity, even in the few patients in whom the initiation of follicular maturation was observed.
4. Finally, it was remarkable to observe that the normal hormonal profile was completely restored during the immediate posttreatment cycle.

Comment

Our data show that the gestodene–ethinyl estradiol combination, when correctly administered in healthy women, effectively inhibits the hypophysial-ovarian axis, thus providing absolute contraceptive security despite the low dosage of the components.

REFERENCES

1. Spona J, Huber J. Pharmacological and endocrine profiles of gestodene. Int J Fertil 1987;32(suppl):6-14.
2. Stickland J, Tolowinska IY, Anthony F, Dennis KJ. The suppression of ovarian function by the new oral contraceptive—Femodene. Contraception 1987;35:447-56.
3. Rabe T, Runnebaum B, Grunwald K, et al. Metabolic effects of a gestodene-containing low-dose oral contraceptive pill. In: Breckwoldt M, Düsterberg B, eds. Gestoden, a new direction in oral contraception. London: Parthenon Publishing, 1988:31-46.
4. Düsterberg B, Hassan SH, Matthes H. Plasma concentrations of gestoden and endogenous sexual hormones in a 21-day treatment cycle with the new oral contraceptive and in two further treatment cycles with defined 2-day breaks in treatment in 6 young women. Arch Gynecol 1985; 237(suppl):67.

Effects of oral contraceptives on lipid metabolism

Jan A. Gevers Leuven, MD, PhD, Marianne C. Dersjant-Roorda, Frans M. Helmerhorst, MD, PhD, Rinse de Boer, MD, Angela Neymeyer-Leloux, MD, and Louis M. Havekes, PhD

Leiden and Hertogenbosch, The Netherlands

A randomized, comparative study of two low-dose oral contraceptives was carried out in 60 nonpregnant women for 12 cycles. Twenty-eight women took a preparation containing 150 μg of desogestrel and 30 μg of ethinyl estradiol; 32 took 75 μg of gestodene and 30 μg of ethinyl estradiol. Baseline values in the two groups were in good agreement. During the first three cycles, serum cholesterol levels remained unchanged, but triglyceride and high-density lipoprotein cholesterol levels increased significantly and to the same extent (about 20%). Low-density lipoprotein cholesterol levels decreased slightly. Apolipoproteins A_1 and A_2 and low-density lipoprotein B increased significantly and similarly. Except for continued increases in low-density lipoprotein B, no further changes were noted during subsequent cycles. We concluded that the two contraceptives have the same impact on lipid metabolism, with all lipid and apolipoprotein values remaining within the normal range. The changes with both pills reflect a mild estrogenic dominance. Implications for coronary artery atherosclerosis appear to be minimal. (Am J Obstet Gynecol 1990;163:1410-3.)

Key words: Atherosclerosis, desogestrel, ethinyl estradiol, gestodene, lipid metabolism, oral contraceptives

From the Gaubius Institute, The Netherlands Organization for Applied Scientific Research, and Academic Hospital, and Groot Zieken Gasthuisd.
Reprint request: Jan A. Gevers Leuven, Gaubius Institute TNO, P.O. Box 612, 2300 AP Leiden, The Netherlands.
6/0/23637

Increased levels of low-density and high-density lipoprotein cholesterol are associated with a high and low incidence of ischemic heart distress, respectively.[1, 2] Low-density lipoprotein cholesterol can cause the disease. Evidence that high-density lipoprotein cho-

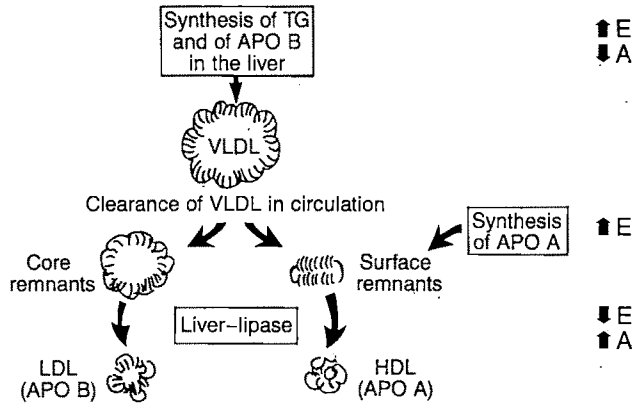


Fig. 1. Simplified scheme of lipoprotein metabolism (*TG*, Triglyceride; *APO*, apolipoprotein; *E*, estrogen; *A*, androgen; *VLDL*, very low-density lipoprotein; *LDL*, low-density lipoprotein; *HDL*, high-density lipoprotein; ↑, enhancement; ↓, inhibition) (From Gevers Leuven JA, Dersjant-Roorda MC, Helmerhorst FM, de Boer R, Neymeyer-Leloux A, Havekes L. AM J OBSTET GYNECOL 1990;163:361.)

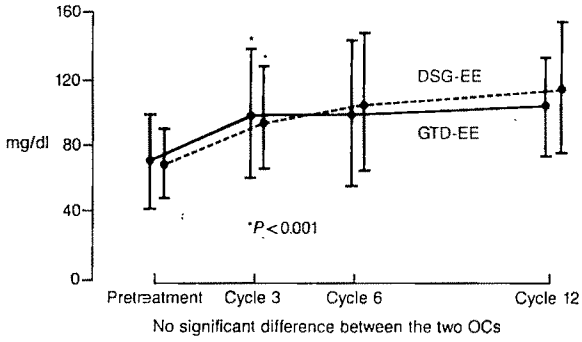


Fig. 2. Mean triglyceride values (± standard deviation) before and during use of contraceptives. (*DSG-EE*, 150 μg desogestrel + 30 μg ethinyl estradiol; *GTD-EE*, 75 μg gestodene + 30 μg ethinyl estradiol.)

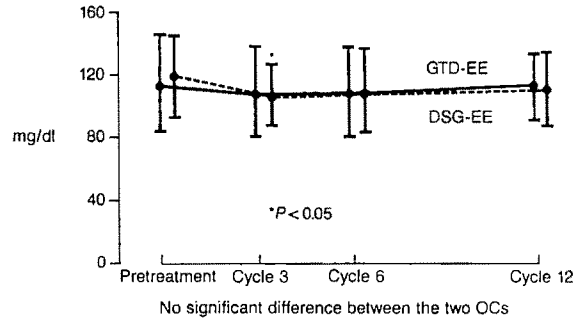


Fig. 3. Mean low-density lipoprotein cholesterol values (± standard deviation) before and during the use of oral contraceptives. (*GTD-EE*, 75 μg gestodene + 30 μg ethinyl estradiol; *DSG-EE*, 150 μg desogestrel + 30 μg desogestrel + 30 μg ethinyl estradiol.)

lesterol is causally associated with an inhibition of atherogenesis is less complete.

Fig. 1 is a simplified scheme of lipoprotein metabolism.[3] In the liver triglyceride synthesis is enhanced by estrogen and inhibited by androgen. This triglyceride is brought into the circulation in particles called very low-density lipoproteins, so these particles are filled with endogenous triglyceride. The synthesis rate of its carrier protein, apolipoprotein B, is also enhanced by estrogen.

In the circulation very low-density lipoproteins are cleared by fat-consuming tissues (such as the heart muscle) and stored in adipose tissue. When triglyceride is removed, the particles shrink, so core remnants are smaller than very low-density lipoproteins. They are brought into the circulation again. These core remnants still contain apolipoprotein B. Liver lipase finally converts them to low-density lipoproteins. The low-density lipoprotein still contains the same amount per particle of apolipoprotein B.

In contrast, surface remnants that are also formed during the clearance of very low-density lipoproteins enter the circulation and are converted into high-density lipoproteins. Estrogen enhances the synthesis of their major carrier protein, apolipoprotein A. Liver lipase is probably involved in the removal of cholesterol from high-density lipoproteins. Lipolytic activity of liver lipase is inhibited by estrogen and enhanced by androgen. Progestogens may have antiestrogen and androgen side effects. When a contraceptive pill containing an estrogen and a progestogen is introduced, as in the study described herein, the resultant effect is not predictable.

Material and methods

A fuller discussion of this study is contained in an article published previously in this JOURNAL.[3] The study enlisted 60 healthy, nonpregnant women between the ages of 20 and 35 years. Most had not previously used an oral contraceptive and none had used one within

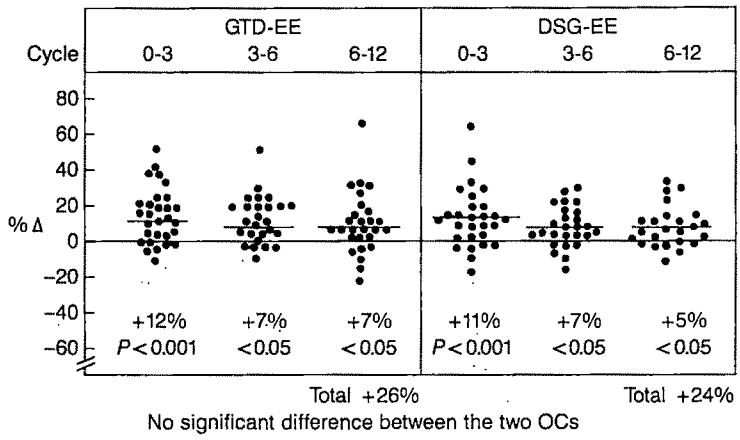


Fig. 4. Individual variations in low-density lipoprotein apolipoprotein B levels from pretreatment to treatment cycles 3, 6, and 12. (*GTD-EE*, 75 μg gestodene + 30 μg ethinyl estradiol; *DSG-EE*, 150 μg desogestrel + 30 μg ethinyl estradiol.) (From Gevers Leuven JA, Dersjant-Roorda MC, Helmerhorst FM, de Boer R, Neymeyer-Leloux A, Havekes L. AM J OBSTET GYNECOL 1990;163:360.)

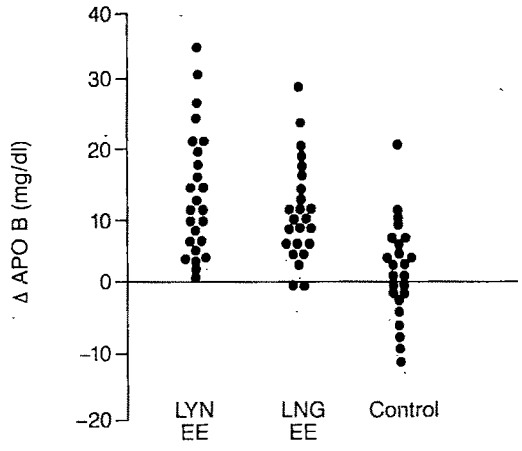


Fig. 5. Effect of two oral contraceptives on apolipoprotein B. (*LYN-EE*, 750 μg lynestrenol + 37.5 μg ethinyl estradiol; *LNG-EE*, 150 μg levonorgestrel + 30 μg ethinyl estradiol.)

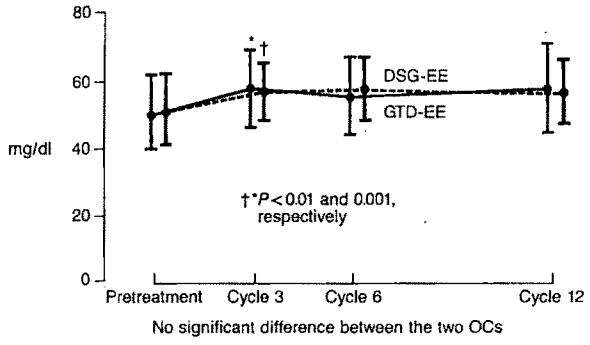


Fig. 6. Mean high-density lipoprotein cholesterol values (± standard deviation) before and during use of oral contraceptives. (*DSG-EE*, 150 μg desogestrel + 30 μg ethinyl estradiol; *GTD-EE*; 75 μg gestodene + 30 μg ethinyl estradiol.)

the past 3 months. The subjects were randomized into two groups: 32 were given 75 μg of gestodene and 30 μg of ethinyl estradiol; 28 women were given 150 μg of desogestrel and 30 μg of ethinyl estradiol. Fasting blood samples were taken between the seventeenth and twenty-fifth day of the pretreatment cycle and between the eighteenth and twenty-second days of cycles 3, 6, and 12. Informed consent was obtained. The committee on medical ethics of the University of Leiden had approved the study.

Results

Triglycerides. When measured pretreatment, triglyceride levels for the two groups were the same (Fig. 2). Through cycle 3, triglyceride levels increased close to the classic level that would be predicted based on the estrogenic effect. After that triglyceride values leveled off.

Low-density lipoprotein cholesterol. Introduction of either oral contraceptive did not lead to an increase in low-density lipoprotein cholesterol (Fig. 3). With the desogestrel-containing combination, there was actually a slight but significant decrease in these levels during the first three cycles.

The carrier lipoprotein of low-density lipoprotein, apolipoprotein B, increased to a significant degree in every period of observation for both contraceptives (Fig. 4). Overall between pretreatment and the twelfth cycle, apolipoprotein B levels increased 26% with the gestodene formulation and 24% with the desogestrel formulation. This is not a significant difference between the two formulations, but the fact that apolipoprotein B levels were increasing was worthy of concern.

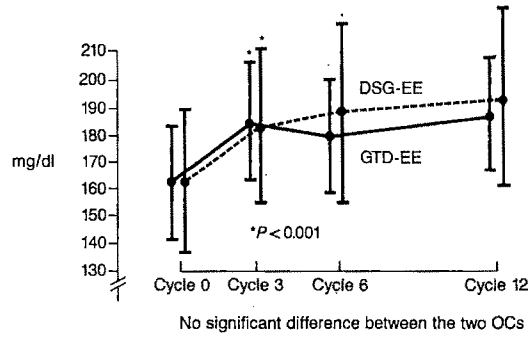


Fig. 7. Mean apolipoprotein A_1 values (± standard deviation) before and during use of oral contraceptives. (*DSG-EE*, 150 μg desogestrel + 30 μg ethinyl estradiol; *GTD-EE*, 75 μg gestodene + 30 μg ethinyl estradiol.)

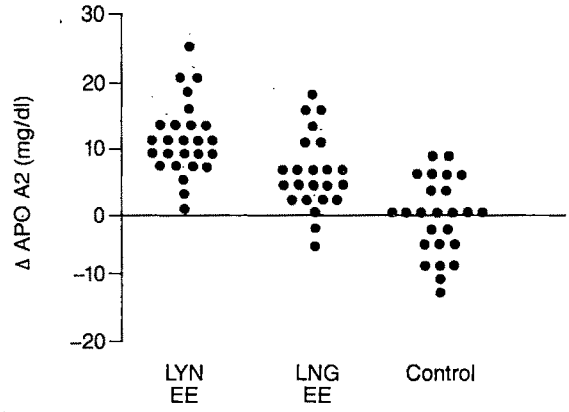


Fig. 8. Effect of oral contraceptives on apolipoprotein A_2. (*LYN-EE*, 750 μg lynestrenol + 37.5 μg ethinyl estradiol; *LNG-EE*, 150 μg levonorgestrel + 30 μg ethinyl estradiol.)

This increase is not unique to these two formulations. When we studied two other combination pills, one containing 750 μg of lynestrenol and 37.5 μg of ethinyl estradiol and the other 150 μg levonorgestrel and 30 μg of ethinyl estradiol, we also observed increases in apolipoprotein B compared with untreated controls (Fig. 5).[4] We suspect the increase in apolipoprotein B is an estrogenic effect.

High-density lipoprotein cholesterol. For both formulations, high-density lipoprotein concentrations increased significantly at a similar rate from pretreatment to cycle 3 (Fig. 6). After cycle 3 the concentrations for both formulations leveled off, albeit with some fluctuation.

Baseline apolipoprotein A_1 values for the formulations were the same, and both showed a comparable, highly significant increase at cycle 3 (Fig. 7). Apolipoprotein A_1 values for the desogestrel combination continued increasing through the balance of the cycles. After a drop between cycles 3 and 6, apolipoprotein A_1 values for the gestodene combination resumed increasing. We only have preliminary data on apolipoprotein A_2 values for these two formulations. However, evidence from our comparison of combination formulations containing lynestrenol or levonorgestrel showed clear increases in apolipoprotein A_2 values over control values (Fig. 8). Apolipoprotein A_2 values were highest in the lynestrenol combination, which contained a larger estrogen component (37.5 μg versus 30 μg ethinyl estradiol).

Comment

From our accumulated evidence to date, we conclude that all mean values of lipoprotein levels increased significantly in users of low-dose oral contraceptives. These changes indicate a mild estrogenic dominance in all four low-dose oral contraceptives we examined in our two studies.

On the basis of current knowledge, it seems unlikely that changes in the estrogen/(antiestrogen + androgen) balance induced by oral contraceptives increase cardiovascular risks. The rise in triglyceride levels is probably of little, if any, relevance.[5] The possible increased risk caused by the rise in apolipoprotein B, which has been observed in studies in men,[6] even if it applies to women, would appear to be offset by the decrease in risk resulting from the increase in apolipoproteins A_1 and A_2.[7]

We thank H. A. Th. de Pagter for her skillful administrative work and P. C. M. de Wit, A. E. M. Kramp, and H. A. van der Voort for skillful technical assistance.

REFERENCES

1. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. New perspectives found in the Framingham Study. Ann Intern Med 1979;90:850-91.
2. Gordon T, Castelli WP, Hjortland MC, et al. High-density lipoprotein as a protective factor against coronary heart disease. Am J Med 1977;62:707-14.
3. Gevers Leuven JA, Dersjant-Roorda MC, Helmerhorst FM, de Boer R, Neymeyer-Leloux A, Havekes L. Estrogenic effect of gestodene or desogestrel containing oral contraceptives on lipoprotein metabolism. Am J Obstet Gynecol 1990 (in press).
4. Gevers Leuven JA, Havekes L, Van de Kooij-Pontier HA, et al. Effect of low-dose oral contraceptives on lipoproteins and lipolytic enzymes: differences between two commonly used preparations. Metabolism 1984;33:1039-42.
5. Hulley SB, Rosenman RH, Bawol RD, et al. Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary heart disease. New Engl J Med 1980;302:878-89.
6. Sniderman A, Shapiro S, Marpole D, Skinner B, Teng B, Kwiterovich PO. Association of coronary atherosclerosis with hyperapobetalipoproteinemia. Proc Natl Acad Sci USA 1980;77:604-8.
7. Avogaro P, Gazzolato G, Bittolo-Bar G, et al. Are apolipoproteins better discriminators than lipids for atherosclerosis? Lancet 1979;1:901-3.

Pharmacokinetics of gestodene and ethinyl estradiol after oral administration of a monophasic contraceptive

Ulrich Täuber, PhD, Wilhelm Kuhnz, PhD, and Michael Hümpel, PhD
Berlin, West Germany

The pharmacokinetic and protein-binding properties of gestodene and ethinyl estradiol have been investigated after single and multiple dosing in several studies in 83 healthy, young women. After oral administration, gestodene is completely absorbed and bioavailable and exhibits dose-linear pharmacokinetics. During long-term pill use, serum levels of gestodene were four to five times higher than after single administration, showing a periodic increase from day 1 to day 10 during each cycle. Ultrafiltration studies revealed that 75.3% of total serum gestodene is bound to sex hormone–binding globulin, 24.1% is bound to albumin, and only 0.6% is not protein bound. Thus gestodene levels during steady state are explained by an increase in sex hormone binding–globulin as a result of concomitant administered ethinyl estradiol and a specific binding of gestodene to this protein. Serum levels of ethinyl estradiol during single and multiple administration were identical and were not different from those observed with another preparation containing 30 μg of ethinyl estradiol. (AM J OBSTET GYNECOL 1990;163:1414-20.)

Key words: Ethinyl estradiol, gestodene, monophasic contraceptive

Progestins and estrogens in oral contraceptives are either drugs, which are, per se, pharmacologically active, or prodrugs, which must be converted into the active compound by the organism. Most progestins used in oral contraception are of the 19-nortestosterone type (Fig. 1). Norethisterone, as the first compound available, is still one of the most widely used progestins and is the active principle of several prodrugs (norethisterone acetate, norethynodrel, ethynodiol diacetate, and lynestrenol).

Changing the methyl residue at C13 to an ethyl group led to norgestrel and its solely active enantiomer levonorgestrel (D (−) norgestrel), whose progestogenic activity is approximately 10-fold higher than that of norethisterone.[1-3] The progestins of the so-called third generation are desogestrel, norgestimate, and the latest development, gestodene. Desogestrel and norgestimate are both prodrugs. Desogestrel is activated in the body by conversion into 3-ketodesogestrel; norgestimate is converted by biotransformation into several metabolites—one, of which the most important is probably levonorgestrel.

After oral administration of a contraceptive pill, a series of events precede or run in parallel while the active ingredients reach their site of action. A necessary prerequisite for drug absorption is the disintegration of the tablet in the gastrointestinal tract and the dissolution of the active ingredients. After absorption and first passage in the liver, the active ingredients are bound to transport proteins in the plasma and are distributed throughout the body by way of the circulation. Parallel to these distribution processes, the compounds are metabolically degraded and excreted with the urine and the bile. Pharmacokinetics describes these processes quantitatively by monitoring drug and metabolite levels in blood and excreta.

This article presents the pharmacokinetic behavior of gestodene in humans.

From the Research Laboratories, Schering AG.
Reprint requests: Ulrich Täuber, PhD, Research Laboratories, Schering AG, Postfach 65 03 11, D-1000 Berlin 65, West Germany.
6/0/23069

Material and methods

Subjects and design of trials. Tables I and II summarize the most important pharmacokinetic studies with gestodene in human volunteers.

Single-dose studies. In the single-dose study by Düsterberg et al., absorption, distribution, biotransformation, and excretion were investigated after intravenous and oral administration of 50 and 500 μg of carbon 14–gestodene (^{14}C-label in the ethinyl group) as a solution in propanediol physiologic saline solution (10/90 v/v) and as micronized substance together with lactose.

In the study by Täuber et al.,[5] basic pharmacokinetic parameters of gestodene, absolute bioavailability, and dose linearity of pharmacokinetics were investigated in a fourfold changeover design in six young women. In a third trial plasma levels of ethinyl estradiol were studied in 18 young female volunteers receiving a single dose of Femovan (gestodene, 75 μg + ethinyl estradiol, 30 μg; Schering Ag, Berlin, West Germany) and Marvelon (desogestrel, 150 μg + ethinyl estradiol, 30 μg; Organon, Oss, The Netherlands) in a crossover design.[6] Both preparations contained 30 μg of ethinyl estradiol.

Multiple-dose studies. Unlike most other drugs,

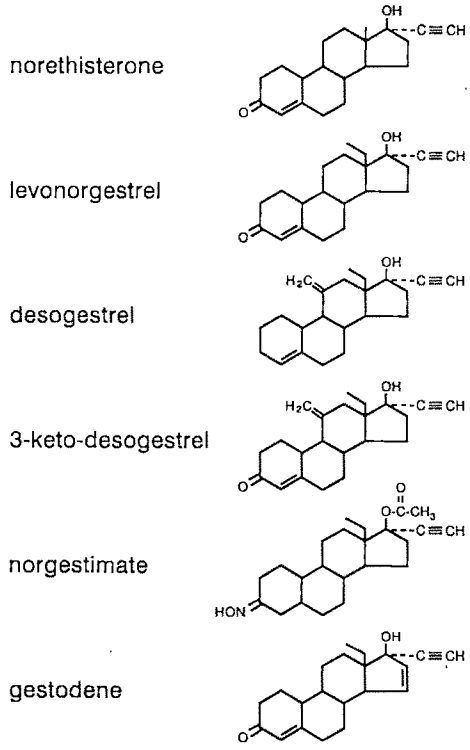


Fig. 1. Chemical structures of progestins used in oral contraception.

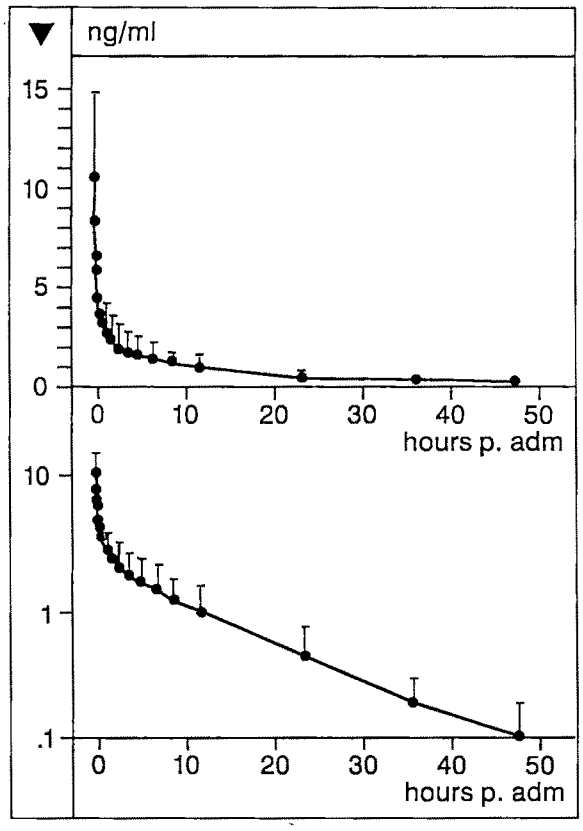


Fig. 2. Plasma gestodene levels after intravenous administration of 75 μg of gestodene to six women (mean ± SD). *Upper figure:* Linear scale; *lower figure:* semilog scale. (From Taüber U, Tack JW, Matthes H. Contraception 1989;40:461-79, Reprinted with permission of Butterworth Publishers.)

plasma levels of 19-nortestosterone–type progestins during multiple dosing cannot be predicted from single-dose pharmacokinetics. This is due to the induction of sex hormone–binding globulin (SHBG) by the concomitant administration of ethinyl estradiol and specific binding of 19-nortestosterone–type progestins to SHBG.[1]

In three studies the plasma or serum levels of gestodene and also those of SHBG and cortisol-binding globulin have been followed over longer time spans.[7-9] In the last study the protein-binding characteristics of progestins were also analyzed, comparing gestodene with 3-ketodesogestrel.[9]

Assays. Total concentrations of gestodene and ethinyl estradiol in plasma or serum were determined after diethyl ether extraction by specific radioimmunoassays developed in the research laboratories of Schering AG.[5, 10] SHBG, cortisol-binding globulin, and cortisol levels were measured with commercial kits.[8]

Free fraction, albumin, and SHBG-bound proportions of sex steroids were determined after spiking of ex vivo samples with ^{3}H-gestodene, ^{3}H-ketodesogestrel, or ^{3}H–ethinyl estradiol (specific radioactivity >1.5 TBq/mmol) by means of an ultrafiltration method similar to that described by Kuhnz et al.[9] and Hammond et al.[11]

Results

Gestodene: Single-dosing. Distribution and elimination processes can be studied undisturbed by absorption phenomena only after intravenous administration. After single intravenous administration of 75 μg as solution, the concentration of gestodene in the plasma declined in three different phases for which half-lives of 10 ± 7 minutes, 1.5 ± 1.4 hours, and 10 ± 2.3 hours have been calculated (Fig. 2). First and second disposition half-lives mainly reflect distribution processes; the terminal half-life reflects the elimination of the drug from the plasma. Gestodene is highly bound to plasma proteins. The apparent volume of distribution of the central compartment (V_C) was determined as 0.15 ± 0.06 L/kg. The volume of distribution during the terminal phase (V_Z) was 0.66 ± 0.43 L/kg.[5]

Gestodene is almost entirely metabolized before it leaves the body. Less than 1% of the dose has been found unchanged in the urine. As with many other steroids, gestodene is metabolized into a large number of metabolites. Biotransformation pathways comprise reduction processes at the 3-keto group as well as at the Δ^4-double bond, hydroxylation at various points of the molecule (including C1, C6, and C11), and conjugation (Fig. 3). By cochromatography of a metabolite isolated from dog urine, it was estimated that <4% of the dose was excreted in the form of a deethinylated,

Table I. Single-dose pharmacokinetic studies with gestodene and ethinyl estradiol in humans

Type of study	*Dose (μg)*		*Route of administration*	*Pharmaceutical formulation*	*No. of subjects*	*Aim of study*	*Reference*
	Gestodene	*Ethinyl estradiol*					
Twofold crossover with ^{14}C-ethinyl-GTD	50 500		IV PO	Propylene glycol Saline 10/90 v/v Capsule with lactose	3	^{14}C-radioactivity in plasma and excreta Balance Metabolite pattern in plasma and urine	Düsterberg et al.[4]
Fourfold crossover	75		IV	Propylene glycol Saline 10/90 v/v	6	GTD plasma levels Pharmacokinetic parameters Absolute bioavailability Dose dependency	Täuber et al.[5]
	25	30	PO	Tablet			
	75	30	PO				
	125	30	PO				
Twofold crossover comparison							
Femovan	75	30	PO	Coated tablet	18	Serum levels of EE	Kuhnz et al.[6]
Marvelon	150*	30	PO	Tablet			

EE, Ethinyl estradiol; *GTD,* gestodene; *IV,* intravenous; *PO,* by mouth.
*Desogestrel.

Table II. Multiple-dose pharmacokinetic studies with gestodene and ethinyl estradiol in humans

Type of study	*Dose (μg)*		*Route of administration*	*Pharmaceutical formulation*	*No. of subjects*	*Aim of study*	*Reference*
	Gestodene	*Ethinyl estradiol*					
Steady-state kinetics over 1 cycle	75	30	PO	Coated tablet	6	GTD, SHBG levels	Düsterberg et al.[4]
Steady-state kinetics over 12 cycles	75	30	PO	Coated tablet	11	GTD, CBG, SHBG, days 1, 10, and 21	Kuhl et al.[12]
Long-term study† Group comparison, day 14 of cycle							
Femovan	75	30	PO	Coated tablet	39	Free and bound GTD, KDG, EE, CBG, SHBG, cortisol	Hümpel et al.[7,8] Kühnz et al.[9]
Marvelon	150*	30	PO	Tablet	30		

CBG, Cortisol-binding globulin; *EE,* ethinyl estradiol; *GTD,* gestodene; *KGD,* 3-ketodesogestrel; *SHBG,* sex hormone–binding globulin; *PO,* by mouth.
*Desogestrel.
†Women had been taking Femovan or Marvelon for a period of 11 ± 5 months or 38 ± 24 months, respectively.

D-ring homoannulated metabolite. The metabolites of gestodene were mainly excreted with the urine with a half-life of approximately 24 hours. Total plasma clearance of gestodene, which represents metabolic clearance, was 0.80 ± 0.53 ml/min/kg (Table III).

After oral administration, gestodene is rapidly absorbed into the systemic circulation. Maximum concentrations are reached after 30 minutes to 3 hours. No absorption problems have been observed after dosage of the tablet formulation. One-hundred percent of the dose reaches the systemic circulation unchanged, as seen from a comparison of areas under the plasma concentration-time curve after oral and intravenous administration; that is, gestodene undergoes no mentionable first-pass metabolization during the absorption process. Plasma concentration-time profiles for gestodene after oral and intravenous administrations are stikingly similar and are essentially superimposable after the absorption process is finished (i.e., 2 to 3 hours after dosing).

The pharmacokinetics of gestodene are dose linear over the whole therapeutic range and cover all dosage forms of both monophasic and triphasic preparations; that is, underlying pharmacokinetic processes obey first-order mechanisms. Plasma levels of gestodene and areas under the plasma level curves increased linearly with the dose, whereas all other pharmacokinetic parameters, such as time of maximum concentration,

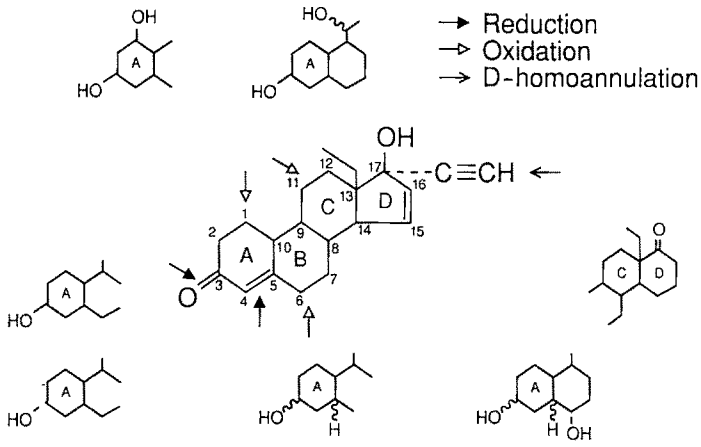


Fig. 3. Biotransformation pathways of gestodene. (Modified from Düsterberg B, Tack JW, Krause W, Hümpel M. Pharmacokinetics and biotransformation of gestodene in man. In: Elstein M, ed. Gestodene: development of a new gestoden low-dose contraceptive. Carnforth, England: Parthenon Publishing, 1987:35-44, by permission.)

Table III. Pharmacokinetic parameters of gestodene after single intravenous administration of 75 μg to six women (mean ± SD)

Parameter	*Result*
First disposition half-life ($t_{1/2}\lambda_1$)	0.16 ± 0.12 hr
Second disposition half-life ($t_{1/2}\lambda_2$)	1.53 ± 1.35 hr
Half-life ($t_{1/2}$)	10.01 ± 2.33 hr
Total plasma clearance (CL)	0.80 ± 0.53 ml/min/kg
Area under curve (AUC)	35.2 ± 15.1 ng · hr/ml
Volume of distribution of central compartment (V_C)	0.15 ± 0.06 L/kg
Volume of distribution during terminal phase (V_Z)	0.66 ± 0.43 L/kg

Data from Täuber U, Tack JW, Matthes H. Contraception 1989;40:461-79, Reprinted with permission of Butterworth Publishers.

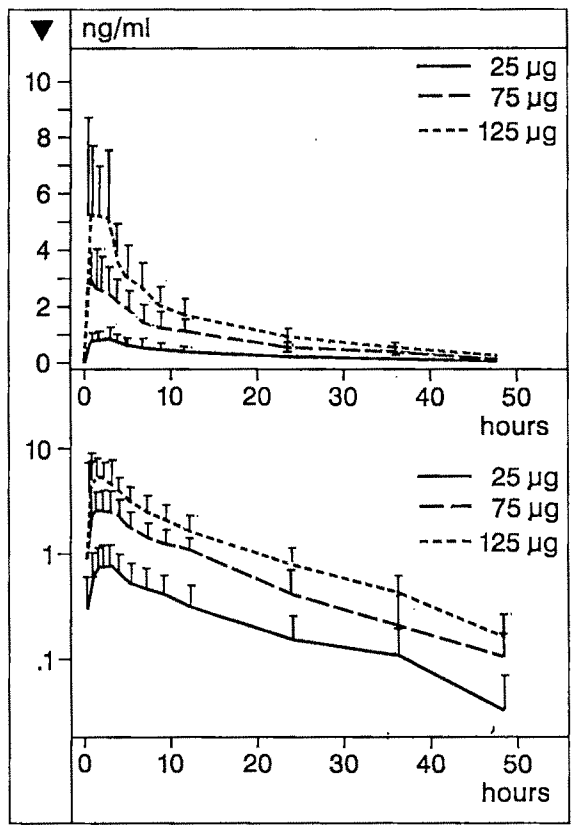


Fig. 4. Plasma gestodene levels after oral administration 25, 75, and 125 μg to six women (mean ± SD). *Upper figure,* linear scale; *lower figure,* semilog scale. (From Taüber U, Tack JW, Matthes H. Contraception 1989;40:461-79, Reprinted with permission of Butterworth Publishers.)

half-lives, volumes of distribution, clearance rate, and bioavailability, were independent of the dose (Fig. 4 and Table IV).[5]

Usually there is only one binding protein for drugs; that is, albumin, which is available in high concentrations in plasma or serum. Certain basic drugs are bound to the α_1-acidic glycoprotein. In 19-nortestosterone progestins but not in C21-progestins, another binding protein, the so-called SHBG that physiologically binds testosterone and estradiol, plays an important role.

Intake of ethinyl-containing oral contraceptives can lead to a gradual increase in SHBG concentration in the plasma. The extent of the SHBG increase depends on the estrogen-progestogen balance of a preparation, that is, on the dose of ethinyl estradiol and on the dose and nature of the progestin. Table V shows the nonprotein-bound fraction and the albumin- and SHBG-bound fraction of gestodene in women with basal (77 ± 28 nmol/L) and induced (186 ± 57 nmol/L) SHBG levels. A 2.4-fold increase in SHBG levels led to a decrease in the free fraction of gestodene, from 1.9% ± 0.3% to 0.6% ± 0.1%. This change in the

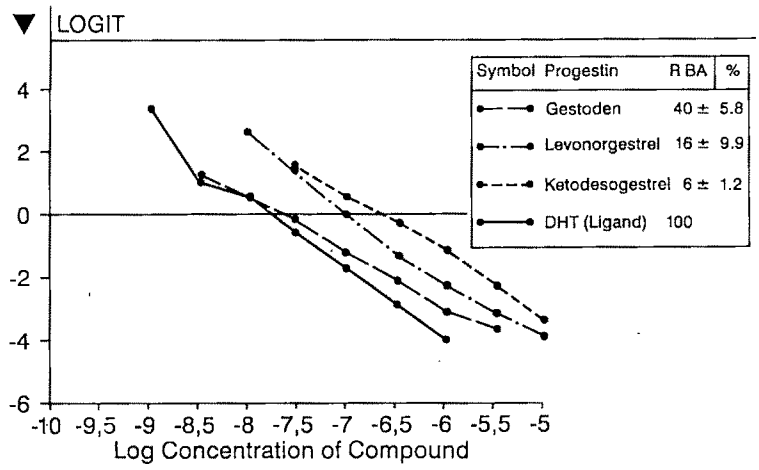


Fig. 5. Replacement of ^{3}H-dihydrotestosterone *(DHT)* from human SHBG (serum of pregnant women) by various progestins, $n = 4$. *RBA*, Relative-binding affinity. (Muhn-Seipoldy HP Unpublished results.)

Table IV. Pharmacokinetic parameters of gestodene after single oral administration of 25, 75, and 125 μg as tablets together with 30 μg ethinyl estradiol (mean ± SD, $n = 6$)

Parameter	*Dosage*		
	25 μg	*75 μg*	*125 μg*
Maximum concentration (C_{max}), ng/ml	1.0 ± 0.4	3.58 ± 1.4	6.98 ± 2.2
Time of maximum concentration (t_{max}), hr	1.9 ± 1.2	1.7 ± 1.2	1.40 ± 0.96
Half-life of absorption (A) ($t_{½A}$), hr	1.2 ± 0.65	0.98 ± 0.6	0.92 ± 0.64
Second disposition half-life ($t_{½}\lambda_2$), hr	0.97 ± 0.7	0.82 ± 0.5	1.30 ± 1.10
Half-life ($t_{½}$), hr	13.94 ± 2.9	11.78 ± 2.3	13.87 ± 2.75
Area under curve (AUC), ng · hr/ml	10.6 ± 5.2	35.0 ± 16.6	61.9 ± 21.5
Fraction of dose systemically available (F)	0.88 ± 0.18	0.99 ± 0.11	1.11 ± 0.18

Data from Täuber U, Tack JW, Matthes H. Contraception 1989;40:461-79, Reprinted with permission of Butterworth Publishers.

nonprotein-bound fraction is obviously caused by a redistribution of gestodene in the human body. At low SHBG levels, almost equal proportions were bound to albumin and SHBG, whereas in the induced SHBG state, the proportion bound to SHBG was more than threefold higher than the proportion bound to albumin.

A competition experiment with the serum of pregnant women confirms the results of the ex vivo–binding studies. The serum of pregnant women containing high levels of SHBG was loaded with ^{3}H-dihydrotestosterone as ligand. The replacement of ^{3}H-radioactivity by various progestins was measured as a function of their concentrations. Relative-binding affinity decreased in the order of gestodene > levonorgestrel > 3-ketodesogestrel (Muhn-Seipoldy HP. Unpublished results) (Fig. 5).

Gestodene: Multiple dosing. Oral contraceptives are taken long term in a regimen of a once daily for 3 weeks followed by a pill-free interval of 1 week. Because of the periodic increase of SHBG over each cycle and the specific binding of gestodene to this protein, serum levels of gestodene cannot be predicted by computer simulation from single-dose pharmacokinetic parameters.

Fig. 6 shows the time course of total gestodene trough levels and SHBG levels in a subject receiving the monophasic preparation over one cycle. Both compounds increased in the plasma up to days 8 to 10, at which time steady state was reached. During the intake-free intervals, gestodene but not SHBG achieved pretreatment values, owing to their different half-lives of 18 hours and 10 to 14 days, respectively.[4]

In Fig. 7 average area under the curve values of total and free gestodene serum level curves determined in 11 women on days 1, 10, and 21 in cycles 1, 3, 6, and 12 are presented.[12] The free levels are in a dynamic equilibrium with concentrations at the target tissues. The periodic increases in total gestodene concentrations and area under the curve values during each cycle are higher than the accumulation determined by the half-life and the dosing interval. Increases observed can be easily explained by the periodic increase in SHBG up to 300% above pretreatment levels and the high-specific binding of gestodene to this binding protein.

Ethinyl estradiol. Fig. 8 shows mean time course of

Table V. Binding of gestodene to human plasma proteins in women with basal and induced SHBG levels

	Single administration (n = 18)	*Multiple administration (n = 38)*
Nonprotein bound, % unbound	1.9 ± 0.3	0.6 ± 0.1
Albumin, % bound	47.8 ± 7.5	24.1 ± 0.1
SHBG, % bound	50.3 ± 7.8	75.3 ± 9.1
SHBG concentration (nmol/L)	77 ± 28	180 ± 61

Data from Täuber U, Tack JW, Matthes H. Contraception 1989;40:461-79; and Kuhnz W, Pfeffer M, Al-Yacoub G. J Steroid Biochem 1990;35:313-8.

Table VI. AUC values of ethinyl estradiol after single dose and during long-term use of Femovan and Marvelon in pg · hr/ml

	Single-dose, intraindividual comparison	*Long-term use, group comparison**
Femovan		
AUC (0-4 hr)	308 ± 83 (*n* = 18)	305 ± 115 (*n* = 39)
AUC (0-24 hr)	1002 ± 332† (*n* = 18)	1246 ± 468‡ (*n* = 39)
Marvelon		
AUC (0-4 hr)	315 ± 77 (*n* = 18)	354 ± 139 (*n* = 30)
AUC (0-24 hr)	1001 ± 311† (*n* = 18)	1360 ± 541‡ (*n* = 30)

AUC, Area under the curve.

*Measured per average on day 14 of cycle after an average intake period of 11 (Femovan) and 38 (Marvelon) months.

†AUC calculations based on sampling points, 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours.

‡AUC calculations based on sampling points 0, 0.5, 1, 2, 3, 4, and 24 hours.

ethinyl estradiol levels in a group of 18 women who received Femovan and Marvelon in a randomized crossover design with a washout period of 1 week between both administrations. Area under the curve (0 to 4 hours) values for both preparations were equal and were also equal to those determined after long-term use of both preparations. Area under the curve (0 to 24 hours) values for long-term use (per average day 14 of cycle in both preparations) were approximately 25% to 30% higher than those after single administration (Table VI). This increase is less than that observed by Kaufmann et al.[13] in ethinyl estradiol trough levels of women using oral contraceptives containing levonorgestrel and 30 or 50 μg of ethinyl estradiol, but is in agreement with the accumulation of ethinyl estradiol that was predictable from plasma half-lives of 6 to 8 hours found by most authors for ethinyl estradiol.[14] Likewise, no difference between Femovan and Marvelon was seen in SHBG, cortisol-binding globulin, or cortisol levels.[7, 8]

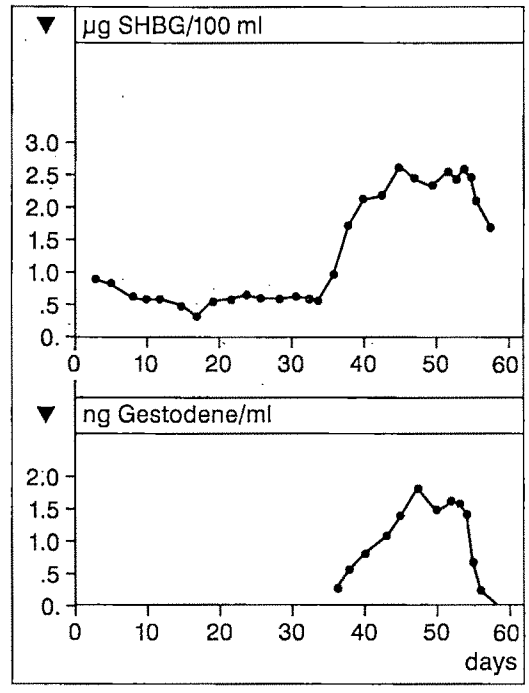


Fig. 6. Plasma levels of SHBG and gestodene as determined for a control cycle and a 21-day treatment cycle (75 μg of gestodene and 30 μg of ethinyl estradiol) in one test subject. (From Düsterberg B, Tack JW, Krause W, Hümpel M. In: Elstein M, ed. Gestodene: development of a new gestoden low-dose contraceptive. Carnforth, England: Parthenon Publishing, 1987:35-44.)

Comment

The monophasic oral contraceptive combining gestodene and ethinyl estradiol provides complete and rapid absorption and bioavailability of gestodene with dose-linear pharmacokinetics. Plasma levels of ethinyl estradiol are no different from those observed with another preparation containing the same amount of ethinyl estradiol.

Periodic increases in the serum levels of gestodene are seen from day 1 through day 8 during each cycle. Gestodene is highly bound to plasma proteins. Slightly more than three fourths of total serum gestodene is bound to SHBG and nearly all the balance to albumin.

REFERENCES

1. Orme ML'E, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1983;8:95-136.
2. Fotherby K. A metabolic assessment of different oral contraceptives. J Obstet Gynecol 1983;3(suppl 2):77-82.
3. Fotherby K. A new look at progestogens. Clin Obstet Gynecol 1984;11:701-22.
4. Düsterberg B, Tack JW, Krause W, Hümpel M. Pharmacokinetics and biotransformation of gestodene in man. In: Elstein M, ed. Gestodene: development of a new gestoden low-dose contraceptive. Carnforth, England: Parthenon Publishing, 1987:35-44.
5. Täuber U, Tack JW, Matthes H. Single dose pharmacokinetics of gestodene in women after intravenous and oral administration. Contraception 1989;40:461-79.

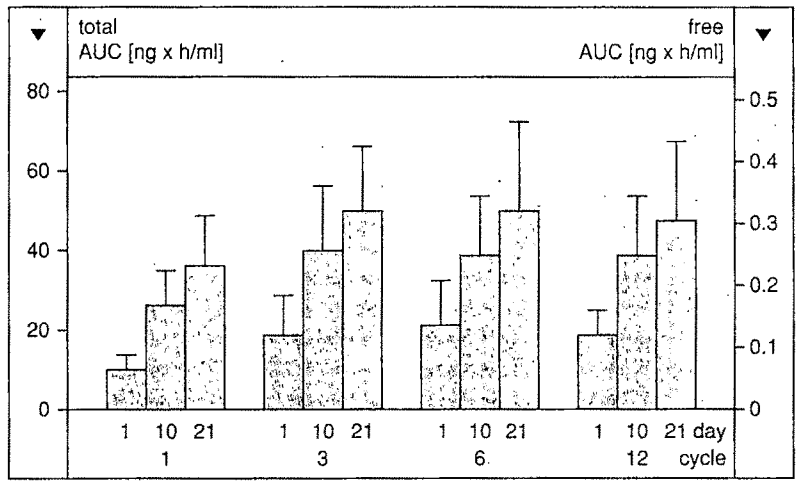


Fig. 7. Areas (0 to 4 hours) under serum level curves of total *(left ordinate)* and free *(right ordinate)* concentration of gestodene on days 1, 10, and 21 of cycles 1, 3, 6, and 12 during intake of monophasic gestodene-coated tablets over a 1-year period (mean ± SD, n = 11). (Total AUC [0-4 hr] values from Kuhl H, Jung-Hoffman C, Heidt F. Contraception 1988;38:477-86.)

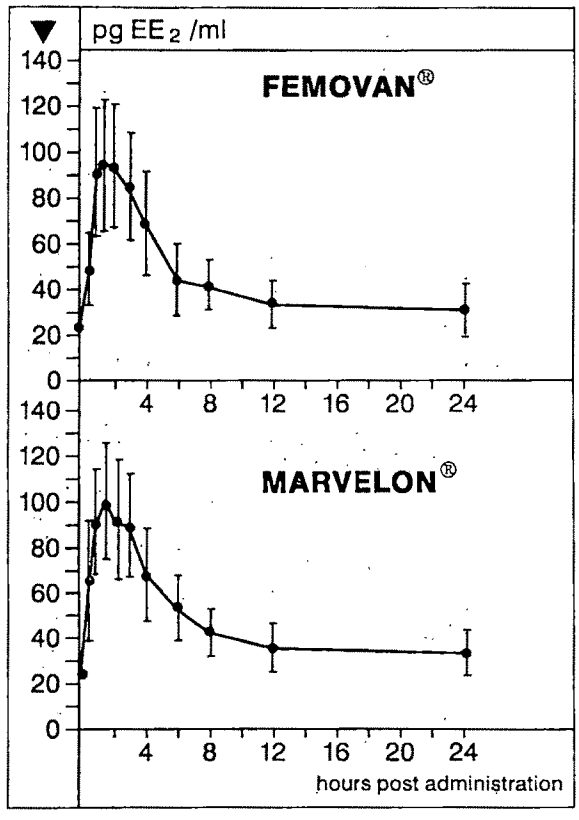


Fig. 8. Plasma levels of ethinyl estradiol in a group of 18 women taking a single dose of either Femovan *(upper figure)* or Marvelon *(lower figure)* in a crossover design (mean ± SD). (From Kuhnz W, Hümpel M, Schütt B, Louton T, Steinberg B, Gansau C. Horm Res 1990;33:40-4. by permission of S. Karger AG, Basel.)

6. Kuhnz W, Hümpel M, Schütt B, Louton T, Steinberg B, Gansau C. Relative bioavailability of ethinylestradiol from two different oral contraceptive formulations after single oral administration to 18 women in an intraindividual crossover design. Horm Res 1990;33:40-4.
7. Hümpel M, Täuber U, Kuhnz W, et al. Protein binding of active ingredients and comparison of serum ethinyl estradiol, sex hormone-binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol biolquivalence from both oral contraceptives. Am J Obstet Gynecol 1990;163:329-33.
8. Hümpel M, Täuber U, Kuhnz W, et al. Comparison of serum ethinyl estradiol, SHBG, CBG and cortisol levels in women using two low-dosed combined oral contraceptives. Horm Res 1990;33:35-9.
9. Kuhnz W, Pfeffer M, Al-Yacoub G. Protein binding of the contraceptive steroids gestodene, 3-ketodesogestrel and ethinylestradiol in human serum. J Steroid Biochem 1990; 35:313-8.
10. Nieuweboer B, Tack J, Täuber U, Hümpel M, Wendt H. Development and application of a radioimmunoassay of the new progestogen gestodene. Contraception 1989;40: 313-23.
11. Hammond GL, Lähteenmäki PLA, Lähteenmäki P, Luukkainen T. Distribution and percentages of non-protein bound contraceptive steroids in human serum. J Steroid Biochem 1982;17:375-80.
12. Kuhl H, Jung-Hoffmann C, Heidt F. Alterations in the serum levels of gestodene and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 75 μg gestodene. Contraception 1988;38:477-86.
13. Kaufmann JM, Thiery M, Vermeulen A. Plasma levels of ethinylestradiol (EE) during cyclic treatment with combined oral contraceptives. Contraception 1981;24:589-602.
14. Newberger J, Goldzieher JW. The pharmacokinetics of ethinylestrogens: a review. Contraception 1977;15:255-84.

16.4.91

November 1990
in two parts, part 1
volume 163, *number* 5

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editors
ALBERT B. GERBIE
STEVEN G. GABBE
THOMAS J. GARITE
MOON H. KIM
ALBERTO MANETTA

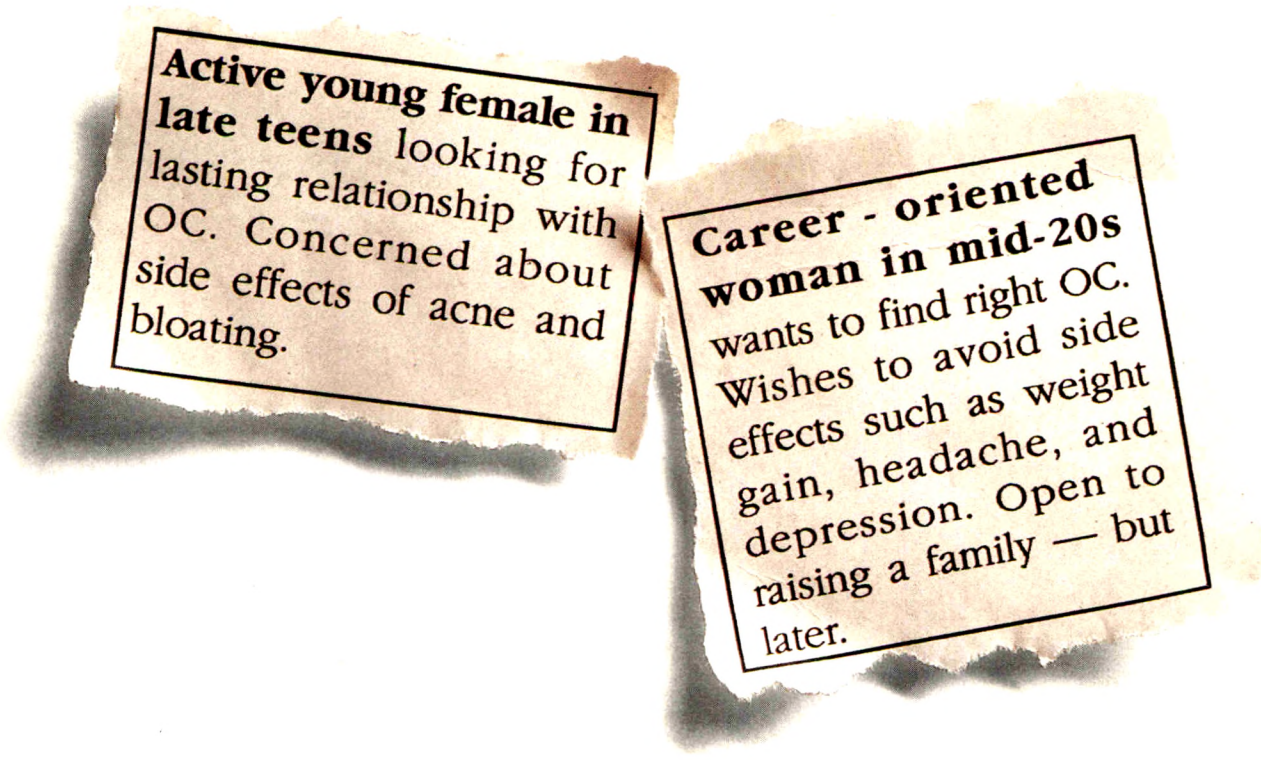
Official Publication

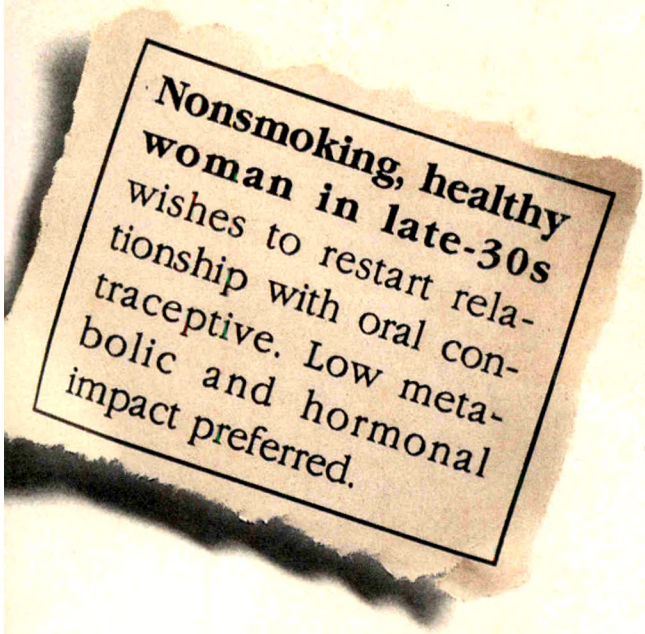
AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

MOSBY-YEAR BOOK, INC.
St. Louis, Missouri 63146-3318

ISSN 0002-9378


Active young female in late teens looking for lasting relationship with OC. Concerned about side effects of acne and bloating.
Career - oriented woman in mid-20s wants to find right OC. Wishes to avoid side effects such as weight gain, headache, and depression. Open to raising a family — but later.



A personalized approach to oral contraceptives.

Because Tri-Norinyl is uniquely phased for all phases of a woman's reproductive life, it's ideal for a wide range of patient types:

Older, nonsmoking patients restarting contraception, because Tri-Norinyl is the only multiphasic with low-impact norethindrone that *lowers* progestin in late cycle to minimize overall hormonal and metabolic impact.

Career-oriented patients delaying childbearing, because reduced progestin helps minimize side effects that can disrupt daily life, such as weight gain, headache, and depression.

Younger patients just starting contraception, because low-impact norethindrone lowers the risk of acne and bloating, and is increased at mid-cycle, to provide enhanced endometrial support when breakthrough bleeding is most likely to occur.

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL®

Uniquely phased for all phases of a woman's reproductive life

Committed to the OB/GYN and women's healthcare

SYNTEX

SYNTEX LABORATORIES, INC
PALO ALTO, CA 94304

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see next page for brief summary of prescribing information.

© 1990 Syntex Laboratories, Inc. 814-H2-172-90

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL®

Uniquely phased for all phases of a woman's reproductive life

ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS**, section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970's—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (> 8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

© 1990 Syntex Laboratories, Inc.
814-H2-170-90

Rev. May 1990

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA. 94304

Mosby Year Book

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1990 by Mosby–Year Book, Inc.

Contents

November
Part 1
1990

CLINICAL SECTION

Clinical Opinion

Human oocyte and preembryo donation: An evolving method for the treatment of infertility 1421

Mark V. Sauer, MD, and Richard J. Paulson, MD

Los Angeles, California

This article reviews the evolution of the technology currently used in preembryo donation and discusses future roles for ovum donation in the assisted reproductive technologies.

Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? 1425

Frank A. Chervenak, MD, and Laurence B. McCullough, PhD

New York, New York, and Houston, Texas

The obstetrician's response to the abortion controversy is ethically analyzed on the basis of responses to the question, "When is the fetus a patient?" and the distinction between professional and private conscience.

Clinical Articles

Interleukin-1β, human leukocyte antigen HLA-DRα, and transforming growth factor-β expression in endometrium, placenta, and placental membranes 1430

Scott Kauma, MD, Dennis Matt, PhD, Stephen Strom, PhD, David Eierman, PhD, and Thomas Turner, BS

Richmond, Virginia

Interleukin-1β, human leukocyte antigen HLA-DRα, and transforming growth factor-β are expressed at the maternal-fetal interface during pregnancy and may regulate the maternal immune response to the fetal semiallograft.

(Contents continued on page 5A)

Vol. 163, No. 5, Part 1, November 1990. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 7351. Annual subscription rates for 1991: domestic, $86.00 for individuals and $150.00 for institutions. Printed in the U.S.A.
Copyright © 1990 by Mosby–Year Book, Inc.

To be continued...



Continuation...an excellent indicator of patient satisfaction.

The Ortho ALL-FLEX* Arcing Spring Diaphragm

- Easy to insert and position
- Easy to clean and maintain
- Easy to use with unscented, colorless, stainless GYNOL II* (2% nonoxynol-9) Contraceptive Jelly
- The combination for continuation—with 4 out of 5 patients continuing after 1 year†

Continuation...it's one reason why physicians continue to make ORTHO ALL-FLEX and GYNOL II the #1 prescribed diaphragm combination in America.‡

Ortho ALL-FLEX*

Arcing Spring

DIAPHRAGM



†Number of patients continuing the method for 12 consecutive months; data on file, Ortho Pharmaceutical Corporation.

‡Data on file, Ortho Pharmaceutical Corporation.

Consult the patient brochure for information on toxic shock syndrome (TSS) and wearing time.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

© OPC 1989 *Trademark OJ-319

ORTHO

Contents continued from page 3A

Prevalence and determinants of estrogen replacement therapy in elderly women 1438

Jane A. Cauley, DrPH, Steven R. Cummings, MD, Dennis M. Black, PhD, Stephen R. Mascioli, MD, and Dana G. Seeley, MS

Pittsburgh, Pennsylvania, San Francisco, California, and Minneapolis, Minnesota

Only a small proportion of elderly women in the United States use estrogen replacement therapy; selection factors for use of estrogen are evident and may introduce bias in studies of estrogen and disease.

Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta 1445

Paul Y. C. Cheung, BSc, John C. Walton, MD, Hsin-Hsiung Tai, PhD, Simon C. Riley, PhD, and John R. G. Challis, PhD, DSc

London, Ontario, Canada, and Lexington, Kentucky

At term, immunoreactive prostaglandin dehydrogenase is localized to trophoblast cells in the human chorion-decidua and to intermediate and syncytiotrophoblast cells in placenta, but the distribution of this immunoreactivity does not change with labor.

The effect of physical activity during pregnancy on preterm delivery and birth weight 1450

Mark A. Klebanoff, MD, MPH, Patricia H. Shiono, PhD, and J. Christopher Carey, MD

Bethesda, Maryland, Los Altos, California, and Oklahoma City, Oklahoma

Reported physical activity was minimally associated with either preterm delivery or gestational age–adjusted birth weight in a prospective cohort of 7101 women.

(Contents continued on page 6A)

1991 Annual subscription rates	U.S.A.	*International (surface mail)* *All regions*	*International (airmail)** *Canada*	*Mexico*	*Region 1*	*Region 2*	*Region 3*
Institutions†	$150.00	$182.00	$215.00	$223.00	$238.25	$290.50	$342.50
Individuals‡	$ 86.00	$118.00	$151.00	$159.00	$174.25	$226.50	$278.50
Students, residents‡	$ 40.00	$ 72.00	$105.00	$113.00	$128.25	$180.50	$232.50

Subscription rates include supplements. Single copies are $8.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

**Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Contents continued from page 5A

Prenatal diagnosis of laryngeal atresia 1456
William J. Watson, MD, John M. Thorp, Jr., MD, Richard C. Miller, MD, Nancy C. Chescheir, MD, Vern L. Katz, MD, and John W. Seeds, MD
Chapel Hill, North Carolina

Ultrasonographic findings in a fetus with laryngeal atresia included enlarged echogenic lungs, compressed heart, and hydrops with ascites and scalp edema.

Correlations between antepartum maternal metabolism and newborn behavior 1458
Thomas Rizzo, PhD, Norbert Freinkel, MD, Boyd E. Metzger, MD, Roger Hatcher, PhD, William J. Burns, PhD, and Peter Barglow, MD
Chicago, Illinois

Poorer antepartum maternal glucoregulation correlated with poorer ratings in three of four dimensions of neonatal neurobehavioral status in pregnancies complicated with diabetes mellitus.

Antenatal microbiologic and maternal risk factors associated with prematurity 1465
James A. McGregor, MDCM, Janice I. French, CNM, MS, Ruth Richter, CNM, MSN, Amalia Franco-Buff, MS, Anne Johnson, MS, Sharon Hillier, PhD, Franklyn N. Judson, MD, and James K. Todd, MD
Denver, Colorado, and Seattle, Washington

Presence of bacterial vaginosis, vaginal *Mycoplasma hominis,* and prior preterm birth was associated with preterm labor in a prospective study of 202 pregnant women.

The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants 1474
J. Studd, M. Savvas, N. Waston, T. Garnett, I. Fogelman, and D. Cooper
London, England

A significant increase in bone density was seen in postmenopausal women receiving subcutaneous estradiol and testosterone that correlated with the serum estradiol level.

Fetal eye movement assessed with real-time ultrasonography: Are there rapid and slow eye movements? 1480
Naoki Horimoto, MD, Takashi Koyanagi, MD, Shoji Satoh, MD, Toshiyuki Yoshizato, MD, and Hitoo Nakano, MD
Fukuoka, Japan

Rapid and slow eye movements were evident during the eye movement period in the human fetus at 33 weeks' gestation and afterward.

(Contents continued on page 9A)

Cefotan arithmetic.

CEFOTAN IV 2g, q12h + doxycycline IV or PO 100 mg, q12h

·CDC·
CENTERS FOR DISEASE CONTROL
RECOMMENDED FOR PID[7]

+ more cost-effective than cefoxitin[1,2]

− less frequent dosing than cefoxitin[3,4]

× several times the half-life of cefoxitin[5,6]

÷ single-dose prophylaxis/ twice-daily treatment



Count on

CEFOTAN® IM/IV
(cefotetan disodium)

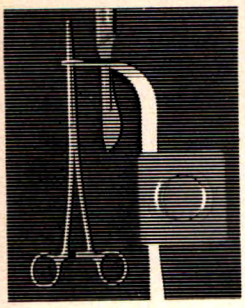
In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

CALCUTTA CENTRAL LIBRARY

© 1989 ICI Americas Inc.



Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.
Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.
In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.
NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).
The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.
If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.
CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.
WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.
Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.
Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.
Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.
PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.
In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.
CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.
INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.
DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.
DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.
As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.
CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.
Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be

developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.
Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.
USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.
USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.
PEDIATRIC USE: Safety and effectiveness in children have not been established.
ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.
DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.
IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.
Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.
When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.
HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.
1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

Manufactured for

STUART PHARMACEUTICALS
A business unit of ICI Americas Inc.
Wilmington, Delaware 19897 USA

Rev. I 11/88

63985-01

Contents continued from page 6A

Continuous or interrupted fascial closure: A prospective evaluation of No. 1 Maxon suture in 402 gynecologic procedures 1485
James W. Orr, Jr., MD, Pamela F. Orr, RN, Jeffrey M. Barrett, MD, John R. Ellington, Jr., MD, Ralph H. Jennings, Jr., MD, Keith B. Paredes, MD, Bruce Patsner, MD, and Dale L. Taylor, MD
Lakeland, Florida

There was no clinical or statistical difference in acute or long-term wound complications when continuous and interrupted abdominal fascial closures were compared.

Endometrioma of the liver 1490
Vittorio Rovati, MD, Edoardo Faleschini, MD, Paolo Vercellini, MD, Giovanni Nervetti, MD, Giorgio Tagliabue, MD, and Guido Benzi, MD
Milan, Italy

A patient with associated endometrioma of the liver and left ovary is described.

Antinuclear antibodies in sera of patients with recurrent pregnancy wastage 1493
Ling Xu, MD, Victoria Chang, BS, Ana Murphy, MD, John A. Rock, MD, Marian Damewood, MD, William Schlaff, MD, and Howard A. Zacur, MD, PhD
Baltimore, Maryland

Women who experience explained or unexplained repeated pregnancy losses have comparable rates of antinuclear antibody–positive sera exceeding those of pregnant and nonpregnant women.

Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels 1497
Paul G. Kratzer, MD, PhD, and Robert N. Taylor, MD, PhD
San Francisco, California

Human corpus luteum activity was correlated with the rate of change of human chorionic gonadotropin and did not appear to be correlated with any other serum factors.

Hemoperitoneum as a result of coital injury without associated vaginal injury 1503
Sterling W. McColgin, MD, Lisa M. Williams, MD, Timothy L. Sorrells, MD, and John C. Morrison, MD
Travis Air Force Base, California, and Jackson, Mississippi

Five cases of hemoperitoneum associated with coital injury but without associated vaginal injury are presented and discussed.

Postmortem chorionic villus sampling is a better method for cytogenetic evaluation of early fetal loss than culture of abortus material 1505
Mark Paul Johnson, MD, Arie Drugan, MD, Frederick C. Koppitch III, MS, Wendy R. Uhlmann, MS, and Mark I. Evans, MD
Detroit, Michigan

Postmortem chorionic villus sampling is a safe, rapid, and reliable technique for the karyotypic evaluation of early pregnancy loss.

(Contents continued on page 11A)

THE PERMANENTE MEDICAL GROUP

NORTHERN CALIFORNIA



The Permanente Medical Group, the largest multispecialty group practice in the U.S., is undergoing explosive growth in its Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley. Our rapid increase in membership has created practice opportunities throughout the region for additional BC/BE Obstetricians and Gynecologists.

Our physician-managed group is part of the comprehensive Kaiser Permanente Medical Care Program. As a TPMG physician, you have access to the latest medical technology and resources, the support of colleagues in all subspecialties — and the opportunity to provide excellent health care without the burdens of managing a practice.

Join us in one of these facilities:

QUALITY

STABILITY

LEADERSHIP

GROWTH

SUPPORT

REWARDS

Santa Rosa — Opening new hospital featuring seven LDR's. Ten OB/GYN physicians and five nurse practitioners. Specialties include infertility, colposcopy, laser surgery and hysteroscopy. Call required once every nine nights. In Northern California's wine country, one hour north of San Francisco.

Santa Clara/Milpitas/Sunnyvale — 28 member department includes 3 perinatologists, GYN oncologist and reproductive endocrinologist. Ongoing clinical research. Our academic-oriented facility has an independent fully-accredited OB/GYN residency and serves as a core rotation for Stanford Medical Students. Academic appointments possible.

San Jose/Gilroy — Our 18 OB/GYN physicians average 3,000 deliveries per year and offer a full range of services including infertility, dysplasia and gyn-urology. On call approximately three nights per month. In-house Level II nursery; prenatal service shared with our nearby Santa Clara medical center. Satellite office in Gilroy. Located south of San Francisco and about one hour from the Monterey coast. The surrounding hills create a pleasant environment in which to live and work.

Hayward/Fremont — Full range of OB/GYN services with Level III L&D/ICN, 3000 deliveries yearly. Full time staff includes perinatologist and four neonatologists. Medical center and associated medical office located on east shore of San Francisco Bay with easy access to entire Bay Area.

Walnut Creek — Department of 15 physicians, a perinatologist and seven nurse practitioners. Two physicians on call daily. Teaching responsibilities for third year residents affiliated with the University of California at San Francisco. Clinical appointments at UCSF also available. Expansion plans include a large ICN, and within five years, a completely new hospital.

Martinez/Antioch — Southern California climate, Northern California charm, Bay Area location. Primarily in/outpatient GYN, outpatient OB. Seven physicians and four nurse practitioners in office and hospital practice. Deliver at the Walnut Creek Medical Center. High-risk pregnancy clinic at Martinez. Labor room call once or twice a month.

Vallejo/Fairfield/Napa — Seventeen physicians and four nurse practitioners at these facilities. Approximately 2,900 deliveries performed each year at the Vallejo medical center. Usual call is every eighth night. Affiliated with David Grant Medical Center residency program at Travis Air Force Base. Our area offers diverse lifestyle options, with both recreational and cultural attractions nearby.

San Rafael/Petaluma — OB/GYN services are available at both the San Rafael Medical Center and the new Petaluma medical offices, which opened in January of 1989.

South Sacramento — Eight physicians and four nurse practitioners serve 104,000 members in fast-growing Central Valley area. Planning new family-oriented L&D facilities.

KAISER PERMANENTE
Good People. Good Medicine.

TPMG offers many benefits: scheduled time off with cross-coverage provided by your colleagues, continuing education, malpractice insurance, a substantial retirement program and special arrangements for physicians transferring from established practice. For complete information, call or send CV to: The Permanente Medical Group, Inc., Richmond Prescott, M.D., Physician Recruitment Services, Dept. AJOG-8346, 1814 Franklin, 4th Floor, Oakland, CA 94612. (800) 777-4912. EOE

Contents continued from page 9A

Detection of congenital toxoplasmosis by chorionic villus sampling and early amniocentesis 1511
Walter Foulon, MD, Anne Naessens, MD, Luc de Catte, MD, and Jean-Jacques Amy, MD
Brussels, Belgium

A case of early and rapid prenatal diagnosis of congenital toxoplasmosis by chorionic villus sampling and amniocentesis is described.

The "stuck twin" phenomenon: Ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses 1513
Barry S. Mahony, MD, Charles N. Petty, MD, David A. Nyberg, MD, David A. Luthy, MD, Durlin E. Hickok, MD, and Jack H. Hirsch, MD
Seattle, Washington

Serial amniocenteses may significantly improve the survival rate of twin gestations affected by the "stuck twin" phenomenon but may be associated with complications among survivors.

Twin-twin transfusion syndrome 1522
Michael A. Urig, MD, William H. Clewell, MD, and John P. Elliott, MD
Phoenix, Arizona

Twin-twin transfusion syndrome associated with acute polyhydramnios in one sac and oligohydramnios in the other is associated with nearly 100% mortality for both fetuses; aggressive amniocentesis appears to offer considerable hope for improved outcome.

Pregnancy in a patient with treated Wilson's disease: A case report 1527
Patrick Dupont, MD, Olivier Irion, MD, and François Béguin, MD
Geneva, Switzerland

Evaluation of the patient's condition before conception and adequate chelating treatment throughout gestation should permit a successful pregnancy in a patient with Wilson's disease.

Nature of gonadotropin-releasing hormone self-priming of luteinizing hormone secretion during the normal menstrual cycle 1529
Michael J. Sollenberger, MD, Elisabeth C. Carlsen, MD, Robert A. Booth, Jr., MS, Michael L. Johnson, PhD, Johannes D. Veldhuis, MD, and William S. Evans, MD
Charlottesville, Virginia

Gonadotropin-releasing hormone induces self-priming of luteinizing hormone release by amplifying the mass and rate of luteinizing hormone secretion, both of which are predicted by serum estradiol levels.

The perinatal impact of cocaine, amphetamine and opiate use detected by universal intrapartum screening 1535
Katherine M. Gillogley, MD, Arthur T. Evans, MD, Robin L. Hansen, MD, Steven J. Samuels, PhD, and K.K. Batra, PhD
Sacramento, California

In a universally screened obstetric population, 299 women were identified as using cocaine, amphetamines, or opiates; confounding demographic factors and perinatal outcome are analyzed.

(Contents continued on page 12A)

Contents continued from page 11A

Prenatal diagnosis of fetal intracranial calcifications 1543
Yasutsugu Koga, MD, Masahiko Mizumoto, MD, Yoshinori Matsumoto, MD, Tetsuo Hattori, MD, Shinichi Tanaka, MD, Toshinobu Tanaka, MD, and Seiichiro Fujimoto, MD
Sapporo, Japan

Fetal intracranial calcifications in a case of intrauterine cytomegalovirus infection were clearly visible by cranial computed tomography and magnetic resonance imaging of the fetus.

Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast 1546
Marie R. Rodway, MSc, Basil Ho Yuen, MB, and Peter C. K. Leung, PhD
Vancouver, British Columbia, Canada

Production of estradiol from androstenedione and testosterone in cultured first-trimester human trophoblast was decreased by treatment with 2 mmol/L 8-bromo-cyclic adenosine monophosphate.

A reappraisal of the need for autologous blood donation in the obstetric patient 1551
Robert L. Andres, MD, Kathleen M. Piacquadio, MD, and Robert Resnik, MD
San Diego, California

Autologous donation of blood by the obstetric patient may not be beneficial or cost effective.

The Maylard incision in gynecologic surgery 1554
B. Frederick Helmkamp, MD, and Hans-B. Krebs, MD
Falls Church, Virginia

A prospective study of the transverse muscle-splitting Maylard incision used in 175 gynecologic patients is presented, and the technique is described in detail.

Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level 1558
Eric Jauniaux, MD, Donald Gibb, MD, Gonzalo Moscoso, MD, and Stuart Campbell, FRCOG
London, England

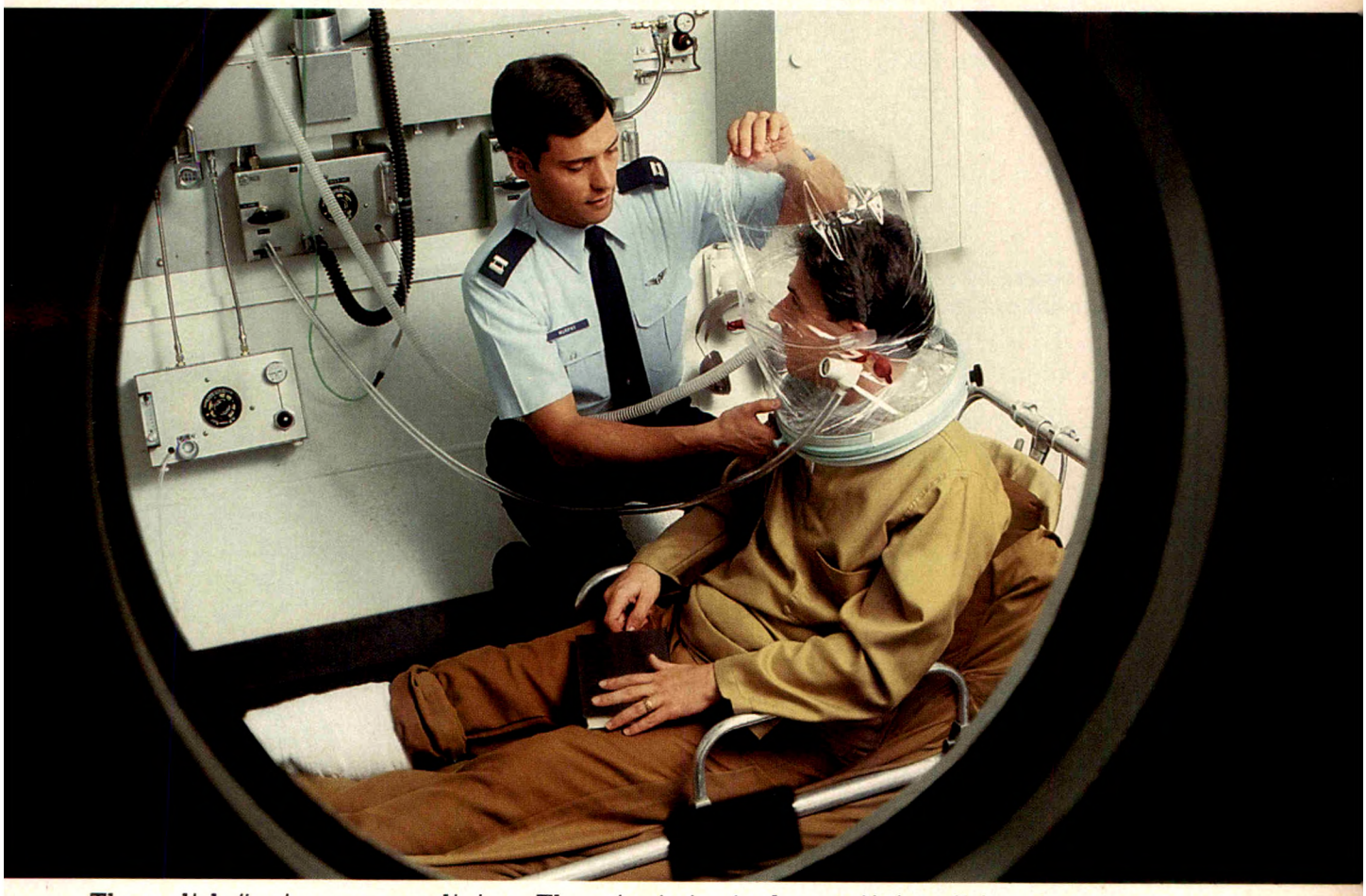
The ultrasonographic features of a developing placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein levels are described.

Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes 1560
Alistair B. Roberts, MD, and Jennifer M. Mitchell, DMU
Auckland, New Zealand

Ultrasonographic measurements of fetal lung length were found to be a good predictor of pulmonary hypoplasia in patients wth prolonged rupture of membranes.

(Contents continued on page 14A)

LAST YEAR, 43 DOCTORS GAVE UP PRIVATE PRACTICE FOR A CHALLENGING NEW CAREER.



They didn't give up medicine. They just started practicing it in a more exhilarating environment.

An environment that affords them many tangible benefits. Like a diverse practice, unlimited access to medical specialities, and financial security. Plus a lot of intangibles. Like a comfortable lifestyle, an opportunity for worldwide experiences and the respect that goes along with being an Air Force officer.

Of course, to enter an environment this rewarding, they did have to give up a few things. Like rent, equipment expenses, malpractice insurance, payrolls and utility bills.

Now, you may be intrigued by such a unique opportunity. But like any good medical professional, you'd probably like a complete case history. So call 1-800-423-USAF. Or send your curriculum vitae to Colonel William E. Patterson, United States Air Force/RSH, Randolph Air Force Base, Texas 78150-5421.

AIM HIGH.

Contents continued from page 12A

Color flow Doppler—A useful instrument in the diagnosis of vasa previa 1566
James A. Harding, MD, David F. Lewis, MD, Carol A. Major, MD, Michael Crade, MD, Jagdish Patel, MD, and Michael P. Nageotte, MD
Long Beach and Orange, California

Color flow Doppler imaging was used to correctly identify vasa previa.

Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants 1568
Marlin D. Mills, MD, Michael P. Nageotte, MD, John P. Elliott, MD, Michael Crade, MD, and Wendy Dorchester, MPH
Phoenix, Arizona, and Long Beach, California

Antenatal ultrasonographic estimate of fetal weight >750 gm and obstetric estimate of gestational age ≥25 weeks predicts improved survival of very-low-birth-weight infants.

Management of selected cystic adnexal masses in postmenopausal women by operative laparoscopy: A pilot study 1574
William H. Parker, MD, and Jonathan S. Berek, MD
Los Angeles, California

Twenty-five postmenopausal women with clinically nonsuspicious cystic adnexal masses were screened with ultrasonography and CA 125; all had benign lesions and most of these were resectable by operative laparoscopy.

Hyperadrenergic orthostatic intolerance as a result of postpartum blood loss 1578
Scott L. Mader, MD, Judith A. Mackall, MD, and Martin P. Emert, MD
Cleveland, Ohio

This report describes orthostatic tachycardia and hypotension caused by protracted postpartum vaginal bleeding that required combination therapy with volume expansion and reduction of sympathetic nervous system activity.

Cervicovaginal microflora and pregnancy outcome: Results of a double-blind, placebo-controlled trial of erythromycin treatment 1580
James A. McGregor, MDCM, Janice I. French, CNM, MS, Ruth Richter, CNM, MSN, Margo Vuchetich, BSN, Vaundeen Bachus, BSN, Kyung Seo, MD, Sharon Hillier, PhD, Franklyn N. Judson, MD, John McFee, MD, Judith Schoonmaker, PhD, and James K. Todd, MD
Denver, Colorado, and Seattle, Washington

A short, defined course of erythromycin was used to examine relationships of cervicovaginal microflora with pregnancy outcomes and preterm birth.

Vitamin E levels in premenstrual syndrome 1591
C. James Chuong, MD, MPH, Earl B. Dawson, PhD, and Edward R. Smith, PhD
Galveston, Texas

Vitamin E deficiency in patients with premenstrual syndrome was not demonstrated in our study.

Contents

Intrauterine *Listeria* infection: Prenatal diagnosis by biophysical assessment and amniocentesis 1596
Robert I. Liner, MD
San Francisco, California

Decreased fetal movement with maternal flulike illness should prompt amniocentesis; in the absence of contractions the cause could still be fetal sepsis as a result of *Listeria.*

Pregnancy outcomes among mothers infected with human immunodeficiency virus and uninfected control subjects 1598
Howard L. Minkoff, MD, Cassandra Henderson, MD, Hermann Mendez, MD, Mitchell H. Gail, MD, PhD, Susan Holman, RN, MS, Anne Willoughby, MD, James J. Goedett, MD, Arye Rubinstein, MD, Pamela Stratton, MD, Judith H. Walsh, and Sheldon H. Landesman, MD
Brooklyn and Bronx, New York, and Bethesda, Maryland

After controlling for confounding variables, we found that children born to 91 women who were human immunodeficiency virus seropositive did not have significantly different birth weights, gestational ages, head circumferences, or Apgar scores than those born to 126 women who were human immunodeficiency virus negative.

Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femur length ratio 1604
Gerald P. Marquette, MD, Marc Boucher, MD, Micheline Desrochers, RN, and Louis Dallaire, MD
Montreal, Quebec, Canada

Ultrasonographic determination of the biparietal diameter/femur length ratio is not a sensitive screening test for trisomy 21.

Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: An accurate and rapid method for detection of fetal chromosome abnormalities 1606
Lee P. Shulman, MD, Avirachan T. Tharapel, PhD, Carole M. Meyers, MD, Owen P. Phillips, MD, Joe Leigh Simpson, MD, and Sherman Elias, MD
Memphis, Tennessee

Direct analysis of cytotrophoblastic cells obtained from second- and third-trimester placentas is an accurate and rapid method for detecting fetal chromosome abnormalities.

Perinatal mortality in Victoria, Australia: Role of group B streptococcus 1609
John R. Fliegner, MD, and Suzanne M. Garland, MD
Parkville and Carlton, Australia

Group B β-hemolytic streptococcus is responsible for >30% of perinatal deaths caused by infection and 1.7% of overall perinatal deaths.

(Contents continued on page 16A)

Contents continued from page 15A

Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: Deviations related to the development of superimposed preeclampsia 1612
Phyllis August, MD, Tomas Lenz, MD, Kathy L. Ales, MD, Maurice L. Druzin, MD, Terri G. Edersheim, MD, J. Milton Hutson, MD, Franco B. Müller, MD, John H. Laragh, MD, and Jean E. Sealey, DSc
New York, New York

The circulating renin-angiotensin-aldosterone system, sex steroids, and renal function were studied in 30 pregnancies of women with chronic hypertension and compared with 58 previously studied normotensive women followed up longitudinally throughout pregnancy.

A comparison of sperm antibody assays 1622
Josef Daru, MD, and Subbi Mathur, PhD
Charleston, South Carolina

Cytotoxicity, Immunobead-binding (immunoglobulin G), and immunofluorescence (immunoglobulin G) sperm antibody assays correlate with each other versus sperm from autoimmune infertile men.

Extensive vulvar and vaginal varicella necessitating abdominal delivery 1630
Cynthia K. Sites, MD, David M. Sherer, MD, David L. Gandell, MD, and James R. Woods, Jr., MD
Rochester, New York

A case is reported in which a patient exposed to chickenpox early in pregnancy received varicella-zoster immune globulin; extensive varicella that developed at term necessitated abdominal delivery.

The role of fetal membranes in regulating production, transport, and metabolism of prostaglandin E_2 during labor 1632
John A. McCoshen, PhD, Dennis R. Hoffman, PhD, Jeremy V. Kredentser, MD, Carolina Araneda, and John M. Johnston, PhD
Winnipeg, Manitoba, Canada, and Dallas, Texas

Increased amniotic prostaglandin E_2 production during labor is not followed by concentration increases on the maternal side of the decidua because of chorion-decidual metabolism of prostaglandin E_2 during transport.

Surgical staples in cesarean section: A randomized controlled trial 1641
Marc G. Villeneuve, MD, Samir Khalifé, MD, Sylvie Marcoux, MD, PhD, and Pierre Blanchet, MD
Sainte-Foy, Quebec, Canada

In a randomized study, the use of the Auto Suture Poly CS 57 disposable surgical stapler for cesarean section hysterotomy reduced subjective blood loss but had no beneficial effect on operating time, postoperative febrile morbidity, or hospital stay.

The associated anomalies that determine prognosis in congenital omphaloceles 1646
Marisa Tucci, MD, and Harry Bard, MD
Montreal, Quebec, Canada

Newborns with giant omphalocele without associated anomalies of the heart, central nervous system, or diaphragm have a good prognosis.

Contents

Current Development

Ultrasonographic assessment of placental abnormalities 1650
Eric Jauniaux, MD, and Stuart Campbell, FRCOG
London, England

The prenatal diagnosis of placental abnormalities by ultrasonography is reviewed, and their pathophysiology and potential clinical implications are discussed.

BASIC SCIENCE SECTION

Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes 1659
Mark A. Plessinger, MS, and James R. Woods, Jr., MD
Rochester, New York

The hypertensive response to cocaine was increased in nonpregnant ewes given progesterone intramuscularly, suggesting that the hormonal environment of pregnancy increases toxicity to cocaine.

Preferential mitogenic activity for myoblast-like cells can be extracted from uterine leiomyoma tissues 1665
Michael Koutsilieris, MD, PhD, Josée Michaud, BSc, and Andreas Nikolis, BSc
Quebec, Quebec, Canada

Extracts of human leiomyoma tissues, in addition to growth substances capable of stimulating or inhibiting the growth of cells with the osteoblast, myoblast, and fibroblast phenotype, contained substance(s) with a preferential activity for myoblast-like cells.

Platelet activating factor enhances in vitro fertilization of rabbit oocytes 1670
William E. Roudebush, PhD, Brijinder S. Minhas, PhD, Deborah D. Ricker, MS, Thomas V. Palmer, BS, and Melvin G. Dodson, MD, PhD
Johnson City, Tennessee

Platelet activating factor treatment of rabbit spermatozoa significantly increases in vitro fertilization rates of cumulus-free oocytes.

Adhesion formation after ovarian wound repair in New Zealand white rabbits: A comparison of ovarian microsurgical closure with ovarian nonclosure 1674
Anne K. Wiskind, MD, Andrew A. Toledo, MD, A. Gatewood Dudley, MD, and Kathryn Zusmanis, BS
Atlanta, Georgia

Microsurgical closure of ovarian surgical defects in New Zealand white rabbits is more adhesiogenic than nonclosure of ovarian wounds.

(Contents continued on page 18A)

Contents continued from page 17A

Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis 1679
Masahiro Sakata, MD, Naoki Terakawa, MD, Takahiro Mizutani, MD, Osamu Tanizawa, MD, Keishi Matsumoto, MD, Nobuyuki Terada, MD, and Katsuichi Sudo, PhD
Osaka, Japan

A combination therapy with danazol and gonadotropin-releasing hormone agonist may be a potential modality in the treatment of advanced endometriosis.

Uterine artery estrogen receptors in the nonpregnant and pregnant guinea pig 1685
Joseph R. Leiberman, MD, Cor C. J. van Vroonhoven, Tech., Ilse Beckmann, PhD, Theo H. van der Kwast, MD, and Henk C. S. Wallenburg, MD, PhD
Beer Sheva, Israel, and Rotterdam, The Netherlands

Estrogen receptors were found in the nuclei of cells belonging to endothelial, muscle, and adventitia layers of the uterine artery wall in the guinea pig.

CONSENSUS REPORT

National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy 1689

LETTERS TO THE EDITORS

Discrepancies in hemoglobin levels Warwick Giles, PhD, Brian Trudinger, MD, and Gavin Wilcox, BSc *Westmead, Australia* 1713

Cytogenetic uncertainties surrounding the fragile X in Martin-Bell syndrome Veena Sindwani, PhD, and Ram S. Verma, PhD *Brooklyn, New York* 1713

Magnesium sulfate is not an ideal anticonvulsant Robert C. Goodlin, MD *Denver, Colorado* 1714

Reply Baha M. Sibai, MD *Memphis, Tennessee* 1715

Parameters of hypothalamic aging David B. Seifer, MD, and Ervin E. Jones, PhD, MD *New Haven, Connecticut* 1715

Reply Sam E. Alexander, MD, and Sezer Aksel, MD *Mobile, Alabama* 1716

Triplet pregnancies—Are we really doing better? Amir Weissman, MD, and Peter Jakobi, MD *Haifa, Israel* 1716

Reply J. E. Milligan, MD *Toronto, Ontario, Canada* 1716

One case of 45,X abortus with high CA 125 level Jerome H. Check, MD *Melrose Park, Pennsylvania* 1717

Contents

ITEMS

Items 1718

INFORMATION FOR AUTHORS

Information for authors 25A

Checklist and copyright statement 29A

Index to advertisers on page 65A

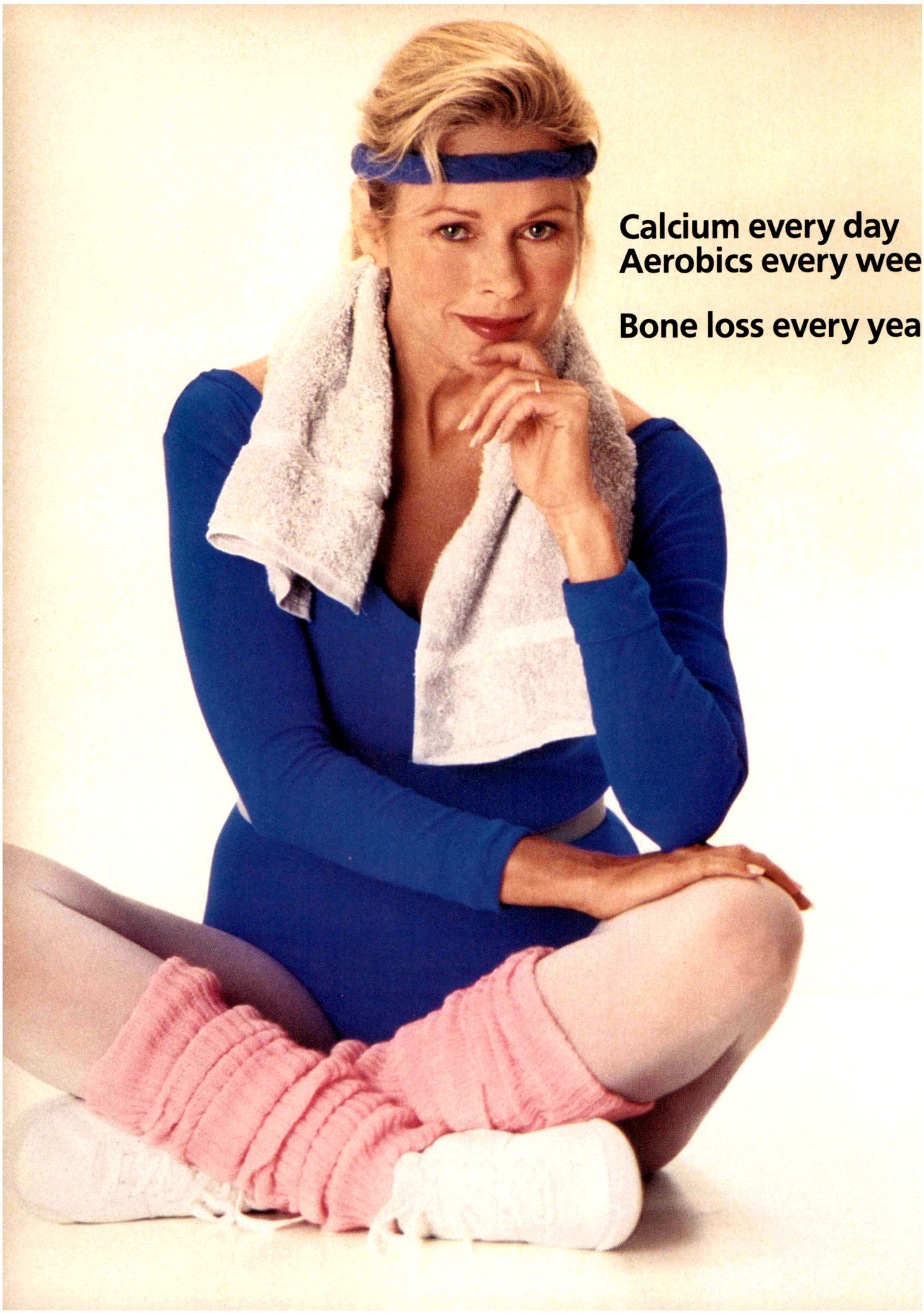
Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

The JOURNAL is abstracted and/or indexed in *Index Medicus, Science Citation Index, Current Contents/Clinical Medicine, Current Contents/Life Sciences, Excerpta Medica, BRS Colleague,* and MEDLINE.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Mosby–Year Book, Inc., for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page per copy is paid directly to CCC, 27 Congress St., Salem, MA 01970; phone (508)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/90 $00.00 + .10.


Calcium every day
Aerobics every wee
Bone loss every yea

She needs PREMARIN to help prevent further bone loss

Calcium[1] and exercise are not enough to prevent post-menopausal osteoporosis, since estrogen deficiency is the primary cause.[2] PREMARIN is the only brand of estrogen indicated to prevent further bone loss and in a recently published study has been shown to reduce the risk of hip fractures by as much as 66%.[3]

Early PREMARIN therapy offers the best protection

The most significant rate of bone loss occurs in early menopause. PREMARIN can help maintain bone mass, preventing the progression of osteoporosis and its consequences.

Protection continues as long as estrogen therapy continues

Clinical studies show that within three years of menopause when therapy is withdrawn, bone loss begins again.[4]

PREMARIN®

(conjugated estrogens tablets)

Proven benefits for menopause and beyond

Estrogens have been reported to increase the risk of endometrial carcinoma in postmenopausal women. Estrogens should not be used in women (or men) with any of the following conditions: known or suspected 1) pregnancy, 2) breast cancer, 3) estrogen-dependent neoplasia, 4) undiagnosed abnormal genital bleeding, 5) active thrombophlebitis or thromboembolic disorders.

See important information on adjacent page.

For moderate-to-severe vasomotor symptoms and for osteoporosis

PREMARIN®
(conjugated estrogens tablets)



0.3 mg 0.625 mg 0.9 mg 1.25 mg 2.5 mg

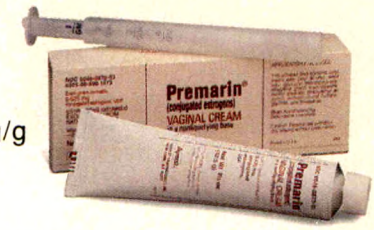
The appearance of these tablets is a trademark of Wyeth-Ayerst Laboratories.

For atrophic vaginitis

PREMARIN®
(conjugated estrogens)

Vaginal Cream

0.625 mg/g



BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA. Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY. Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.
There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

References:
1. Nilas L, Christiansen C, Rødbro P: Calcium supplementation and postmenopausal bone loss. *Br Med J* 1984; 289:1103-1106. **2.** Riggs BL: Research Directions in Osteoporosis. Scientific Workshop, National Institutes of Health, February 9-11, 1987. **3.** Kiel DP, Felson DT, Anderson JJ, et al: Hip fracture and the use of estrogens in postmenopausal women. *N Engl J Med* 1987;317:1169-1174. **4.** Christiansen C, Christensen MS, Transbøl I: Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. *Lancet* 1981;1:459-461.

WYETH

A Caring Commitment to Female Health

WYETH-AYERST LABORATORIES
Philadelphia, PA 19101

© 1990 Wyeth-Ayerst Laboratories

American Journal of Obstetrics and Gynecology

Editors

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*
FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*
E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*
STEVEN G. GABBE, *Associate Editor*
THOMAS J. GARITE, *Associate Editor*
MOON H. KIM, *Associate Editor*
ALBERTO MANETTA, *Associate Editor*

Advisory committee on policy

Garland Anderson	Walter S. Keifer	Frank Miller
William R. Anderson	M. Tucker Laffitte, Jr.	Daniel R. Mishell, Jr.
Mason C. Andrews	Emmet J. Lamb	John C. Morrison
E. Forrest Boyd	Roger A. Lobo	Karl C. Podratz
Robert C. Cefalo	James M. Maharry	Ruth W. Schwartz
Donald R. Coustan	L. Russell Malinak	James R. Scott
A. Jerome Freeland	Douglas J. Marchant	Henry A. Thiede
Norman F. Gant	Arthur M. McCausland	Carol Trumbold
W. Glenn Hurt	James A. Merrill	Ronald V. Wade

Board of corresponding editors

Luigi Carenza, *Rome, Italy*
T. K. A. B. Eskes, *Nijmegen, The Netherlands*
Wolfgang Künzel, *Giessen, West Germany*
Matsuto Mochizuki, *Kobe, Japan*
M. Reuben Peyser, *Tel Aviv, Israel*
Nils-Otto Sjöberg, *Malmö, Sweden*
Gordon M. Stirrat, *Bristol, England*
Claude Sureau, *Paris, France*
Carl Wood, *Melbourne, Victoria, Australia*
Olavi Ylikorkala, *Helsinki, Finland*

Publisher
MOSBY-YEAR BOOK, INC., 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*

American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover the Journal is the official publication of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY

GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

CLEVELAND SOCIETY OF OBSTETRICIANS AND GYNECOLOGISTS

American Journal of Obstetrics and Gynecology

Information for Authors

Copyright © 1990 by Mosby–Year Book, Inc.

Editors

John I. Brewer, *Editor in Chief*
Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
Steven G. Gabbe, *Associate Editor*
Moon H. Kim, *Associate Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
Thomas G. Garite, *Associate Editor*
Alberto Manetta, *Associate Editor*
University of California, Irvine, Medical Center,
Department of Obstetrics and Gynecology, 101 The
City Drive, South, Building 26, Orange, CA 92668

Sherry A. Merryman, *Editorial Assistant*
Tel.: (714) 634-6572

Publisher
MOSBY-YEAR BOOK, INC., 11830 Westline
Industrial Drive, St. Louis, Missouri 63146-3318

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370 (ext. 7591)

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. Donald R. Coustan, Department of Obstetrics and Gynecology, Women and Infants Hospital of Rhode Island, 101 Dudley St., Providence, RI 02905.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. The specified copyright statement that follows the Information for Authors in each issue of the JOURNAL must be completed, signed by all authors, and submitted with the manuscript. (The Checklist and Copyright Statement may be torn from the JOURNAL or photocopied for submission.) If not completed in full, it will be returned to the author for completion.

Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the

Editors that manuscripts emanating from a particular institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed. The species of nonhuman animals used in an investigation must be named in the title, abstract, and key words of the manuscript.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

Disclaimer. Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus.*

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name,* and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

For *black-and-white illustrations* submit three sets of 3×4 inch (minimum) to 5×7 inch (maximum) unmounted, glossy photographic prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black-and-white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted.

Do not use paper clips or mar the surface of prints in any way.

Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with): author(s) name(s), title of article, number, and top marked clearly.

Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in

sequence after the references. Titles should be included in the legend, *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Letters to the Editors. Letters to the Editors must be submitted in duplicate, typed double-spaced, and the text of the manuscript must be limited to a maximum of 400 words [excluding references, name(s) and address(es) of the signer(s), and the phrase "To the Editors"]. The submitted Letter to the Editors must be accompanied by a signed copyright statement. If there are two or more signers of the Letter to the Editors, one of the authors may sign the copyright statement but must add to the statement the following: "I sign for and accept responsibility for releasing this letter on behalf of any and all co-signers." These requirements apply to all types of Letters to the Editors. Letters should be sent to Dr. Zuspan.

Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints

Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

CHECKLIST AND COPYRIGHT STATEMENT

Both the checklist and the copyright statement forms **must be completed** and sent with each submitted manuscript. If **not** completed, these forms will be returned to the author for completion.

These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

—The original and two copies of the manuscript are submitted.
—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.
—Three sets of unmounted glossy photographic prints are submitted, properly numbered and labeled on the back of each print.
—Three sets of computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back are submitted. None are dot matrix or photographic prints.
—Human experimentation has been approved by the local institution as stated in the Methods section.
—Guidelines for the care and use of animals approved by the institution have been followed as indicated in the Methods section.
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
—All pages are numbered in the following order: title page, condensation, abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
—Signed, written permission from both the copyright holder and the original author for the use of tables, figures, or quotations previously published and their complete references are enclosed with the manuscript.
—Signed, written permission for the use of quotations of personal communications and unpublished data has been obtained from the person(s) being quoted and is enclosed.

Authorship

—In the covering letter that accompanies the submitted manuscript I/we have confirmed that all authors fulfilled both conditions required for authorship.

Conflict of interest

—In the covering letter that accompanies the submitted manuscript the commercial association of the author or of any co-authors that might pose a conflict of interest is described.

Previous publications

—Enclosed with the submitted manuscript are two reprints each of articles the author or co-authors have previously published or have in manuscript form dealing with same patients, same animals, same laboratory experiment, or same data, in part or in full, as those reported in the submitted manuscript. Further explanation is provided in the covering letter that accompanies the submitted manuscript.

Title page

—These elements are given in the following sequence, and are typed double-spaced.
—Title
—Author(s) name(s) and highest academic degree(s)
—City(ies), state(s), and country other than U.S. in which the study was conducted are given
—The name(s) of the institution(s) section(s), division(s), department(s) in which the study was performed is provided and the author(s) institutional affiliation at the time of the study is indicated
—Acknowledgment of financial support is cited
—Name, address, and business and home telephone numbers of author to whom requests for reprints are to be sent
—Reprints will not be available. This has been stated on the title page
—Since the corresponding author is different from the author to whom reprint requests are to be sent, his/her name, address, and business and home telephone numbers have been added
—At the bottom of the page, a running head not exceeding 52 characters (including word spaces) has been typed

Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract

—The abstract is typed double-spaced with required margins (on page 3) headed by the title and the author(s) name, not exceeding 150 words. For case reports and brief communications the limit of 50 words is not exceeded.
—Three to five key words or short phrases are placed beneath the abstract on page 3.

Tables

—Each table headed by a caption and numbered in Roman numerals is typed double-spaced on a separate page.
—Tables are cited in sequence in the text.

Figures

—Are numbered in Arabic numerals and are cited in numerical sequence in the text.

—Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

References

—Are typed double-spaced.

—Are numbered consecutively in the order they are cited in the text.

—The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors were followed.

—Abstracts, personal communications, and unpublished work are not used as numbered references but are mentioned in the text with the written approval of the person being quoted and the signed approval is enclosed.

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

COPYRIGHT STATEMENT

Must be signed by ALL authors

"The undersigned author(s) transfers all copyright ownership of the manuscript entitled

to Mosby–Year Book, Inc., in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author."

The responsible author(s) must be named_____ _____
(Type name) Signature

Each author's name must be typed underneath the signature.

_____ _____

_____ _____

_____ _____

_____ _____

Date: _____

Fellowships

The American Board of Obstetrics and Gynecology, Inc.: Approved fellowship training programs in obstetrics and gynecology subspecialties: Gynecologic Oncology; Reproductive Endocrinology; Maternal-Fetal Medicine

CATEGORY State: City Institution	Number of approved positions	Director of program
GYNECOLOGIC ONCOLOGY		
Alabama: Birmingham		
Univ Ala	1 per year (total 2)	Edward E. Partridge
California: Los Angeles		
LAC/USC Med Ctr	1 per year (total 2)	C. Paul Morrow
UCLA	1 per year, alt 2 per year (total 3)	Jonathan S. Berek
California: Orange		
Univ Calif Irvine	1 alt 2 alt 1 (total 4, alt 5) (3 year program)	Michael Berman
Connecticut: New Haven		
Yale Univ	1 per year (total 2)	Peter E. Schwartz
DC: Washington		
Georgetown Univ	1 per year (total 2)	Gregorio Delgado
Walter Reed Army Med Ctr	1 per year (total 2)	Robert C. Park
Florida: Miami		
Univ Miami	1 per year, alt 2 per year (total 3)	Hervy E. Averette
Florida: Tampa		
Univ So Fla	1 per year (total 2)	Denis Cavanagh
Iowa: Iowa City		
Univ Iowa Hosp & Clinics	1 per year (total 2)	Barrie Anderson
Kentucky: Lexington		
Univ Ky	1 per year (total 2)	John R. van Nagell, Jr.
Maryland: Baltimore		
Johns Hopkins Univ	1 per year (total 2)	John L. Currie
Massachusetts: Boston		
Brigham & Women's Hosp	1 per year (total 2)	Ross Berkowitz
Mass Gen Hosp	1 per year (total 2)	Arlan F. Fuller
Michigan: Ann Arbor		
Univ Mich	1 per year (total 2)	James A. Roberts
Minnesota: Minneapolis		
Univ Minn	1 per year, alt 2 per year (total 3)	Leo B. Twiggs
Minnesota: Rochester		
The Mayo Clinic	1 per year (total 3) (3 year program)	Karl C. Podratz
Missouri: St Louis		
Washington Univ	1 per year (total 2)	Ming-Shian Kao
New York: Brooklyn		
SUNY Health Sci Ctr/Brooklyn	1 alt years (total 1)	John G. Boyce
New York: Buffalo		
Roswell Park Cancer Inst	1 per year (total 2)	M. Steven Piver
New York: New York		
Memorial Sloan-Kettering	2 per year (total 4)	John L. Lewis, Jr.
Mt Sinai Med Ctr	1 per year (total 2)	Carmel J. Cohen
New York: Rochester		
Univ Rochester	1 per year (total 2)	Brent DuBeshter

Fellowships are listed alphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
GYNECOLOGIC ONCOLOGY—Cont'd		
North Carolina: Chapel Hill		
Univ NC	1 per year (total 2)	Wesley C. Fowler, Jr.
North Carolina: Durham		
Duke Univ	1 per year (total 2)	Daniel L. Clarke-Pearson
Pennsylvania: Hershey		
Milton S. Hershey Med Ctr	1 per year (total 2)	Rodrigue Mortel
Pennsylvania: Philadelphia		
Hosp Univ Pa	1 per year (total 2)	John J. Mikuta
Texas: Dallas		
Univ Tex/Southwestern	1 per year (total 3) (3 year program)	Roberto Yazigi
Texas: Houston		
M. D. Anderson Cancer Center	3 per year (total 6)	J. Taylor Wharton
REPRODUCTIVE ENDOCRINOLOGY		
California: La Jolla		
Univ Calif San Diego Med School	2 per year (total 4)	Samuel S. C. Yen
California: Los Angeles		
LAC/USC Med Ctr	3 per year (total 6)	Rogerio A. Lobo
UCLA/Cedars-Sinai Med Ctrs	2 per year (total 4)	Howard L. Judd
California: Orange		
Univ Calif Irvine Med Ctr	1 per year (total 2)	Ricardo H. Asch
California: San Francisco		
Univ Calif San Francisco	3 per year (total 6)	Robert B. Jaffe
Connecticut: Farmington		
Univ Conn	1 per year (total 2)	Anthony A. Luciano
Connecticut: New Haven		
Yale Univ	3 per year, alt 4 per year (total 7)	Alan H. DeCherney
DC: Washington		
George Washington Univ	1 per year (total 2)	Robert J. Stillman
Georgetown Univ School Med	1 per year (total 2)	James A. Simon
Walter Reed Army Med Ctr	3 per year (total 6)	Thomas A. Klein
Georgia: Augusta		
Med Coll Ga	2 per year (total 4)	Paul G. McDonough
Illinois: Chicago		
Michael Reese Hosp	2 per year (total 4)	Edward L. Marut
Northwestern Univ	1 per year (total 2)	Anne Colston Wentz
Rush Med Coll	2 per year (total 4)	Ewa Radwanska
Univ Chicago	1 alt years (total 1, alt 2) (3 year program)	James R. Schreiber
Iowa: Iowa City		
Univ Iowa	1 per year (total 2)	F. K. Chapler
Kentucky: Lexington		
Univ Ky	1 per year (total 2)	Ken Muse, Jr.
Kentucky: Louisville		
Univ Louisville	1 per year (total 2)	Marvin A. Yussman
Maryland: Baltimore		
Johns Hopkins Univ School Med	3 per year (total 6)	John A. Rock
Univ Md	1 per year (total 2)	Eli Y. Adashi

Fellowships are listed alphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
REPRODUCTIVE ENDOCRINOLOGY—Cont'd		
Massachusetts: Boston		
Brigham & Women's Hosp	3 per year (total 6)	Kenneth J. Ryan
Michigan: Detroit		
Wayne State Univ	1 per year, alt 2 per year (total 3)	Kamran S. Moghissi
Minnesota: Minneapolis		
Univ Minn	1 per year (total 2)	George E. Tagatz
Minnesota: Rochester		
The Mayo Clinic	1 per year (total 2)	Steven J. Ory
Missouri: St. Louis		
Washington Univ	1 per year (total 2)	Ronald C. Strickler
New Jersey: New Brunswick		
UMDNJ/Robt Wood Johnson	1 alt years (total 1)	Ekkehard Kemmann
New Jersey: Newark		
UMDNJ/New Jersey Med School	2 per year (total 4)	Cecilia L. Schmidt
New York: New York		
Coll Phys Surg/Columbia Univ	1 per year (total 2)	Raphael Jewelewicz
Mt. Sinai Med Ctr	1 per year (total 2)	J. Victor Reyniak
NY Hosp/Cornell Univ	1 per year, alt 2 per year (total 3)	Zev Rosenwaks
North Carolina: Chapel Hill		
Univ NC	1 per year (total 2)	Mary G. Hammond
North Carolina: Durham		
Duke Univ	1 per year, alt 2 per year (total 3)	Arthur F. Haney
Ohio: Cincinnati		
Univ Cincinnati	1 per year (total 2)	James H. Liu
Ohio: Columbus		
Ohio State Univ	1 per year, alt 2 per year (total 3)	Moon H. Kim
Oregon: Portland		
Oregon Health Sci Univ	1 per year (total 2)	Kenneth A. Burry
Pennsylvania: Philadelphia		
Hosp Univ Pa	3 per year (total 6)	Luigi Mastroianni
Tennessee: Memphis		
Univ Tenn	1 per year (total 2)	John E. Buster
Texas: Dallas		
Univ Tex/Southwestern	2 per year, alt 3 per year (total 5)	Bruce R. Carr
Texas: Houston		
Baylor Coll Med	2 per year (total 4)	William E. Gibbons
Texas: San Antonio		
Univ Tex/San Antonio	1 per year (total 2)	Robert S. Schenken
Vermont: Burlington		
Univ Vt	1 per year (total 3) (3 year program)	Mark Gibson
Virginia: Charlottesville		
Univ Va	1 alt years (total 1)	James D. Kitchin III
Virginia: Norfolk		
Eastern Va Med School	2 per year (total 4)	Charles C. Coddington
Wisconsin: Madison		
Univ Wis	1 per year (total 2)	Sander S. Shapiro
Canada: Montreal (Que)		
McGill Univ	1 per year (total 2)	A. Brian Little

Fellowships are listed alphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE		
Alabama: Birmingham		
Univ Ala	1 per year, alt 2 per year (total 3)	John C. Hauth
Arizona: Tucson		
Univ Ariz	1 per year (total 2)	Kathryn L. Reed
Arkansas: Little Rock		
Univ Ark	1 per year (total 2)	J. Gerald Quirk, Jr.
California: La Jolla		
Univ Calif San Diego Med Ctr	1 per year, alt 2 per year (total 3)	Robert Resnik
California: Loma Linda		
Loma Linda Univ	1 per year (total 2)	Barry Block
California: Los Angeles		
King-Drew Med Ctr	1 per year (total 2)	Milton I. Lee
LAC/USC Med Ctr	4 per year (total 8)	Richard H. Paul
UCLA	1 per year (total 2)	Brian J. Koos
California: Orange		
Univ Calif Irvine/Long Beach Hosp	3 per year (total 6)	Thomas J. Garite
California: San Francisco		
Univ Calif San Francisco	4 per year (total 8)	Julian T. Parer
California: Torrance		
Harbor-UCLA/Cedars-Sinai	3 per year (total 6)	Michael Ross
Colorado: Denver		
Univ Colo	2 per year (total 4)	Roger L. Lenke
Connecticut: Farmington		
Univ Conn	2 per year (total 4)	Anthony M. Vintzileos
Connecticut: New Haven		
Yale Univ	1 per year (total 2)	John C. Hobbins
DC: Washington		
George Washington Univ	1 per year (total 2)	John H. Grossman III
Georgetown Univ	1 per year (total 2)	John T. Queenan
Florida: Gainesville		
Univ Fla	1 per year (total 2)	Amelia C. Cruz
Florida: Miami		
Univ Miami	2 per year (total 4)	Mary Jo O'Sullivan
Florida: Tampa		
Univ So Fla Coll Med	3 per year (total 6)	William F. O'Brien
Georgia: Augusta		
Med Coll Ga	1 per year (total 2)	Lawrence D. DeVoe
Illinois: Chicago		
Northwestern Univ Med Ctr	2 per year, alt 3 per year (total 5)	Michael L. Socol
Rush Presbyterian/St Lukes	1 per year (total 2)	Howard T. Strassner, Jr.
Univ Chicago	1 per year (total 2)	Mark Phillippe
Univ Ill	2 per year (total 4)	Andre Bieniarz
Illinois: Springfield		
Southern Ill Univ	1 per year (total 2)	Kofi S. Amankwah
Indiana: Indianapolis		
Indiana Univ School Med	1 per year (total 2)	Alan M. Golichowski
Iowa: Iowa City		
Univ Iowa	1 per year (total 2)	Carl P. Weiner
Kentucky: Louisville		
Univ Louisville School Med	2 per year (total 4)	Joseph A. Spinnato
Louisiana: New Orleans		
La State Univ	1 per year (total 2)	Harvey A. Gabert

Fellowships are listed alphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE—Cont'd		
Maryland: Baltimore		
Johns Hopkins Univ	2 per year (total 4)	Timothy R. B. Johnson
Univ Md	1 per year, alt 2 per year (total 3)	David A. Nagey
Massachusetts: Boston		
Brigham & Women's Hosp	2 per year (total 4)	Fredric D. Frigoletto, Jr.
Tufts Univ/St Margaret's	2 per year (total 4)	Mary E. D'Alton
Michigan: Ann Arbor		
Univ Mich Med Ctr	1 per year (total 3) (3 year program)	Robert H. Hayashi
Michigan: Detroit		
Wayne State Univ	2 per year (total 4)	Sidney F. Bottoms
Mississippi: Jackson		
Univ Miss	2 per year (total 4)	John C. Morrison
Missouri: St Louis		
Washington Univ Med Ctr	3 per year (total 6)	Roy H. Petrie
New Jersey: New Brunswick		
UMDNJ/Robt. Wood Johnson	1 per year (total 2)	John T. Harrigan
New Jersey: Newark		
UMDNJ/NJ Med School	1 per year (total 2)	Joseph J. Apuzzio
New Mexico: Albuquerque		
Univ NM	2 per year (total 4)	Luis B. Curet
New York: Bronx		
Albert Einstein Coll Med	2 per year, alt 3 per year (total 5)	Irwin R. Merkatz
New York: Brooklyn		
SUNY Health Sci Ctr/Brooklyn	1 per year (total 2)	Howard L. Minkoff
New York: Buffalo		
SUNY Health Sci Ctr/Children's Hosp	1 per year (total 2)	William P. Dillon
New York: New York		
Columbia Univ	2 per year (total 4)	Harold E. Fox
Mt Sinai Med Ctr	1 per year, alt 2 per year (total 3)	Richard L. Berkowitz
NY Hosp/Cornell Univ	1 per year (total 2)	Maurice L. Druzin
NY Univ	1 per year (total 2)	Bruce K. Young
New York: Rochester		
Univ Rochester	1 per year, alt 2 per year (total 3)	James R. Woods, Jr.
New York: Stony Brook		
SUNY Health Sci Ctr/Stony Brook	1 per year (total 2)	David A. Baker
New York: Valhalla		
NY Med Ctr/Westchester Cnty	1 per year (total 2)	Nergesh Tejani
North Carolina: Chapel Hill		
Univ NC	1 per year (total 2)	John W. Seeds
North Carolina: Durham		
Duke Univ	1 per year, alt 2 per year (total 3)	Allen P. Killam
North Carolina: Winston-Salem		
Bowman Gray School Med	1 per year (total 2)	J. C. Veille
Ohio: Cincinnati		
Univ Cincinnati	2 per year (total 4)	Tariq A. Siddiqi
Ohio: Cleveland		
Cleveland Metro Gen Hosp	2 per year (total 4)	LeRoy J. Dierker, Jr.
Hosp Cleveland/MacDonald Hosp	2 alt years (total 2)	Method Duchon
Ohio: Columbus		
Ohio State Univ	1 per year (total 2)	Jay D. Iams
Oklahoma: Oklahoma City		
Univ Ok Col Med	1 per year (total 2)	Gary R. Thurnau

Fellowships are listed alphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE—Cont'd		
Oregon: Portland		
Ore Health Sci Univ	1 per year (total 2)	Richard I. Lowensohn
Pennsylvania: Philadelphia		
Hosp Univ Pa	2 per year (total 4)	Arnold W. Cohen
Pa Hosp	2 per year (total 4)	Ronald J. Bolognese
Thomas Jefferson Univ	1 per year, alt 2 per year (total 3)	Ronald J. Wapner
Pennsylvania: Pittsburgh		
Univ Pittsburgh	2 per year (total 4)	Daniel I. Edelstone
Rhode Island: Providence		
Brown Univ	1 per year (total 2)	Donald R. Coustan
South Carolina: Charleston		
Med Univ SC	1 per year (total 2)	Roger B. Newman
Tennessee: Memphis		
Univ Tenn	2 per year (total 4)	Baha M. Sibai
Tennessee: Nashville		
Vanderbilt Univ	1 per year (total 2)	Frank H. Boehm
Texas: Dallas		
Univ Tex/Southwestern	3 per year, alt 4 per year (total 7)	Larry C. Gilstrap III
Texas: Houston		
Baylor Coll Med	3 per year (total 6)	David B. Cotton
Univ Tex/Houston	3 per year (total 6)	Valerie M. Parisi
Texas: San Antonio		
Univ Tex/San Antonio	2 per year (total 4)	Edward R. Newton
Utah: Salt Lake City		
Univ Utah	1 per year (total 3) (3 year program)	D. Ware Branch
Vermont: Burlington		
Univ Vt	1 per year (total 3) (3 year program)	Eleanor L. Capeless
Virginia: Norfolk		
Eastern Va Med School	1 per year (total 2)	Donald L. Levy
Washington: Seattle		
Univ Wash	1 per year (total 2)	Thomas J. Benedetti
Washington: Tacoma		
Madigan Army Med Ctr	1 per year (total 2)	John A. Read
Wisconsin: Madison		
Univ Wis	1 per year (total 2)	Chester B. Martin, Jr.

Fellowships are listed alphabetically by state.

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 163 *number* 5 *part* 1 NOVEMBER 1990

CLINICAL SECTION

Clinical Opinion

Human oocyte and preembryo donation: An evolving method for the treatment of infertility

Mark V. Sauer, MD, and Richard J. Paulson, MD

Los Angeles, California

The utilization of oocyte and preembryo donation in the treatment of infertility has recently expanded. Since 1983, modifications in the retrieval of donated female gametes have enhanced both the safety and the efficacy of the procedure. Pregnancy rates in women receiving donated preembryos are remarkably high, with success occurring in 25% to 50% of transfer cycles. The technology used for preembryo donation continues to evolve, and the current and future role of ovum donation in the treatment of infertility is expanding. (Am J Obstet Gynecol 1990;163:1421-4.)

Key words: Preembryo donation, donor oocytes, uterine lavage, ovarian failure

Human oocyte and preembryo donation has become a viable medical alternative for treatment of infertility in women with ovarian failure. Approximately 0.3% of reproductive-aged women experience ovarian failure, which until recently represented an irrevocable loss of fertility.[1] However, with sex steroid hormone replacement and preembryo donation, functionally agonadal women now have an excellent chance of becoming pregnant.

The method used in human preembryo donation and its application for the treatment of infertility is relatively new. However, the utilization of this technique has become increasingly commonplace. Although initially directed at producing pregnancies in women with ovarian failure, recent success has also been reported after the transfer of donated preembryos to women with intact ovarian function but who otherwise have a poor fertility prognosis.[2] Summarized in this report are the evolution of preembryo donation in humans and the projected role of this method in infertility therapy.

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Southern California.
Reprint requests: Mark V. Sauer, MD, Women's Hospital, 1240 N. Mission Road, #L946, Los Angeles, CA 90033.
6/1/22991

Past developments

Since the late nineteenth century preembryo donation has been successfully performed in more than a dozen mammalian species. Most commonly, uterine lavage (endometrial washing) has been utilized to retrieve the free-floating conceptus before host implantation. This technique has been used in the breeding of exotic animals and has become increasingly popular in animal husbandry.[3] In the cattle industry the efficiency of this process is enhanced by superovulation of the donor cows, followed by their artificial insemination. In this manner multiple preembryos are obtained with uterine lavage. Preembryos that are retrieved are then individually transferred to multiple recipient animals, thus increasing the number of offspring generated per lavage procedure.[4]

Early attempts of ovum donation in humans utilized preembryo recovery techniques analogous to those used in animals. Between 1980 and 1987, clinical trials using uterine lavage of artifically inseminated fertile female donors resulted in the establishment of 13 clin-

Table I. Summary of early experience using uterine lavage to recover in vivo fertilized ova for preembryo donation

Site	*No. of inseminations*	*No. of lavages*	*No. of ova recovered*	*No. of pregnancies*
University of California, Los Angeles[5]	90	240	40 (44%)	4
University of Pavia[6]	58	58	25 (43%)	9

Table II. Summary of early experience in transferring preembryos to functionally agonadal women using in vivo fertilization of donated oocytes

Investigator	*No. of embryo transfers*	*No. of pregnancies*
Lutjen et al.[8]	31	6 (19% per ET)
Navot et al.[9]	8	2 (25% per ET)
Rosenwaks[10]	32	8 (25% per ET)

ET, Preembryo transfer.

ical pregnancies (Table I).[5,6] At that time uterine lavage represented the only nonsurgical alternative for procuring donated female gametes, since other assisted reproductive technologies, such as in vitro fertilization and gamete intrafallopian transfer, required the donor to undergo general anesthesia and laparoscopy. However, the natural fecundity of even the most fertile female donors limited the retrieval of viable preembryos to approximately 25% of cycles lavaged. Other aspects of this procedure lessened its clinical acceptability, as infection and retained pregnancies were observed in donors.[7]

Concomitant advances in the technology of human in vitro fertilization led to the use of this method for ovum donation. Initial reports of pregnancies in small series of functionally agonadal women using oocytes donated and fertilized in vitro appeared from 1984 through 1987.[8-10] These early attempts required the donor to undergo follicle aspiration surgically, usually by laparoscopy or minilaparotomy. Although the number of cases was small, results were encouraging, with most investigators reporting pregnancy rates of approximately 25% per embryo transfer attempt (Table II). However, the clinical applicability of this approach was limited by the difficulty of recruiting donors to undergo surgery. Furthermore, because of the inherent invasiveness of the follicle aspiration, the Ethics Committee of the American Fertility Society expressed concern that the risk of the procedure to the donor might outweigh the benefits of the procedure to the recipient couple.[11]

However, with time, technologic advances in ultrasonography led to the development of transvaginal ultrasonography-directed follicle aspiration. This simplification of the technique of in vitro fertilization has amounted to a technologic breakthrough for ovum donation. Transvaginal ultrasonography-directed follicle aspiration has proved to be a safe and effective means for retrieving oocytes. Women can now undergo oocyte aspiration without incurring the discomfort and risks of general anesthesia and surgery. Oocyte donors once again have a nonsurgical alternative.

Present status

The in vitro fertilization–embryo transfer registry of the Society of Assisted Reproductive Technology (SART) reported 17 clinics in calendar year 1987[12] and 26 clinics in calendar year 1988 that were performing in vitro fertilization–embryo transfer with donated oocytes.[13] In the 1988 series, 130 patients underwent 158 preembryo transfers. Fifty-one (32%) of the donor transfers produced a clinical pregnancy and 36 (23%) live deliveries resulted, including 12 sets of twins and one set of triplets. These numbers contrast with the 12% live-birth rate reported to SART in the same year for standard in vitro fertilization. There were no chromosomal abnormalities or congenital anomalies reported with this procedure.

A wide range of ethical issues surround donor payment and anonymity. While oocyte donation can be performed in either an anonymous or a directed fashion, most programs have maintained the anonymous approach and use supernumerary oocytes donated by couples undergoing in vitro fertilization. In other cases oocytes are donated by women agreeing to ovarian hyperstimulation before an operative procedure such as tubal ligation. In addition, sisters are often used as oocyte donors. In many cases couples prefer a sibling to an unrelated oocyte donor.[14] As the practice of oocyte donation has become a reality, the demand for donors has increased, resulting in a shortage of available donors and long waiting lists. Accordingly, several clinics have developed a registry of fertile women who agree to undergo ovarian hyperstimulation and follicle aspiration specifically for the purpose of donating all the gametes to the recipient. Again this practice may be performed anonymously.

At the University of Southern California a nonanonymous approach has been preferred.[15] Approximately half of our patients preselected a friend or sibling as a prospective donor before our evaluation. Couples with-

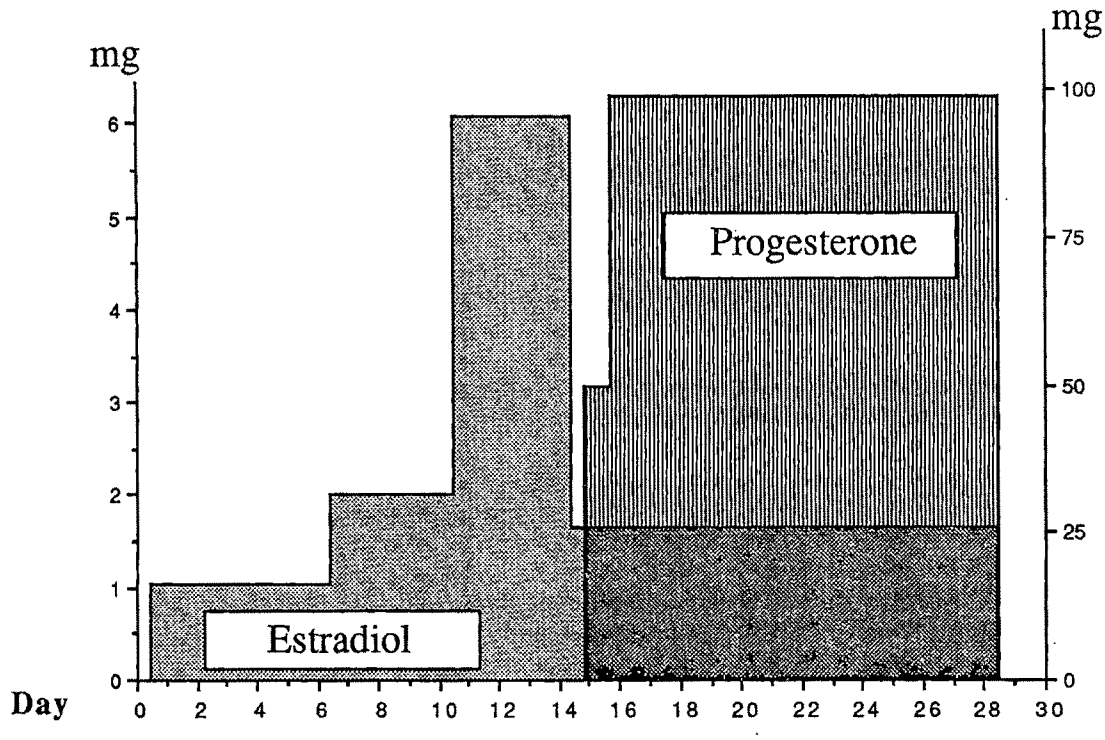


Fig. 1. Depicted is the current hormone replacement regimen used for functionally agonadal recipients of donated preembryos. Oral estradiol and intramuscular progesterone are administered daily in divided doses.

Table III. Summary results for the first 20 functionally agonadal women (aged 24 to 45 years) participating in the oocyte donation program at the University of Southern California

No. of recipients	20
No. of oocytes per recipient per attempt	11.9 ± 1.2 (range 3-25)
Fertilization rate (No. of preembryos per No. of oocytes)	194/326 (60%)
No. of transfer cycles	26
No. of preembryos transferred per transfer cycle	4.5 ± 0.3 (range 1-6)
No. of implantations per transferred preembryo	31/120 (26%)
No. of clinical pregnancies	
Per transfer attempt	16/26 (62%)
Per total participants	16/20 (80%)
No. of ongoing per delivered pregnancies	
Per transfer attempt	12/26 (46%)
Per total participants	12/20 (60%)
No. of multiple gestations in ongoing pregnancies	5/12 (42%)

out a designated donor were matched nonanonymously with one of the several previously screened oocyte donors. This approach has occasionally allowed an individual oocyte donor known to produce large numbers of oocytes an opportunity to provide gametes to more than one recipient couple at a time.[16] A summary of the outcome of our initial 20 patients is listed in Table III.

Future considerations

Refinements of the present methods of preembryo donation will inevitably lead to simplified protocols for hormone replacement of the recipients. The endometria of functionally agonadal women can be successfully prepared by a variety of replacement protocols.[17] Most of these protocols use a combination of oral estradiol and intramuscular progesterone to establish a receptive endometrium. Our replacement regimen is depicted in Fig. 1. Its utilization at the University of Southern California has led to preembryo implantation rates that are threefold higher than those observed in our patients undergoing standard in vitro fertilization.[18] This increased implantation rate has led to higher pregnancy rates. Indeed, pregnancy rates reported with oocyte donation are among the highest

reported for any fertility-enhancing procedure. The unique combination of oocytes obtained from stimulated cycles in fertile women and the optimized endometrial environment in which these preembryos are placed make for these unprecedented results.

Recent reports of successful preembryo transfers to women made artifically agonadal after pituitary down regulation may establish an even greater applicability for donor oocytes.[2] Women with very poor fertility prognoses, such as those with failure of multiple attempts of in vitro fertilization, older women, or those who fail to respond to ovarian hyperstimulation, may ultimately benefit from endometrial preparation followed by oocyte donation.

Preliminary reports of pregnancies after unstimulated donor cycles or in ovaries incidentally removed from women at the time of surgery may further modify the approach to retrieving gametes.[19] Simplifying the procedural aspects of occyte donation should further reduce the present risks of ovarian hyperstimulation and aspiration.

In summary, pregnancy after oocyte and preembryo donation is now a reality and will become more commonplace as we approach the twenty-first century. Ironically, it appears that women who only a decade ago were considered hopelessly infertile may have a higher probability of conception after donor preembryo transfer than women with ovarian function undergoing standard in vitro fertilization.

REFERENCES

1. Aiman J, Smentek C. Premature ovarian failure. Obstet Gynecol 1985;66:9-14.
2. Serhal P, Craft I. Oocyte donation in 61 patients. Lancet 1989;1:1185-7.
3. Anderson G. Embryo transfer in domestic animals. In: Advances in veterinary science and comparative medicine. Long: Academic Press, 1983 vol 29:129-62.
4. Seidel G. Superovulation and embryo transfer in cattle. Science 1981;211:351-8.
5. Sauer M, Bustillo M, Gorrill M, Louw J, Marshall J, Buster J. An instrument for the recovery of preimplantation uterine ova. Obstet Gynecol 1988;71:804-6.
6. Formigli L, Formigli G, Roccio C. Donation of fertilized uterine ova to infertile women. Fertil Steril 1987;47: 162-5.
7. Sauer M, Anderson R, Paulson R. Superovulation of ovum donors undergoing uterine lavage. Fertil Steril 1989;51:131-4.
8. Lutjen P, Trounson A, Leeton J, Findlay J, Wood C, Renou P. The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. Nature 1984; 307:174-5.
9. Navot D, Laufer N, Kopolovic J, et al. Artifically induced endometrial cycles and establishment of pregnancies in the absence of ovaries. N Engl J Med 1986;314:806-11.
10. Rosenwaks Z. Donor eggs: their application in modern reproductive technologies. Fertil Steril 1987;47:895-909.
11. Ethics Committee of the American Fertility Society. Donor eggs in in-vitro fertilization. In: Ethical considerations of the new reproductive technologies. Fertil Steril 1986; 46(suppl 1):42S.
12. Medical Research International, the American Fertility Society Special Interest Group. In vitro fertilization/embryo transfer in the United States: 1987 results from the National IVF-ET Registry. Fertil Steril 1989;51:13-20.
13. Medical Research International, the American Fertility Society Special Interest Group. In vivo fertilization/embryo transfer in the United States: 1988 results from the IVF-ET registry. Fertil Steril 1990;53:13-20.
14. Sauer M, Rodi I, Scrooc M, Bustillo M, Buster J. Survey of attitudes regarding the use of siblings for gamete donation. Fertil Steril 1988;49:721-2.
15. Sauer M, Paulson R, Macaso T, Francis-Hernandez M, Lobo R. Establishment of a nonanonymous donor oocyte program: preliminary experience at the University of Southern California. Fertil Steril 1989;52:433-6.
16. Sauer M, Paulson R, Lobo R. Simultaneous establishment of pregnancies in two ovarian failure patients using one oocyte donor. Fertil Steril 1989;52:1072-4.
17. Navot D, Anderson T, Droesch K, Scott R, Kreiner D, Rosenwaks Z. Hormonal manipulation of endometrial matruation. J Clin Endocrinol Metab 1989;68:801-7.
18. Paulson R, Sauer M, Lobo R. Embryo implantation after human in vitro fertilization: importance of endometrial receptivity. Fertil Steril 1990;53:870-4.
19. Cha K, Yoon S, Ko J, et al. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycle, their culture in vitro and their transfer according to the donor oocyte program [Abstract]. In: Proceedings of the forty-fifth annual meeting of the American Fertility Society, San Francisco, California, November 13-16, 1989.

Does obstetric ethics have any role in the obstetrician's response to the abortion controversy?

Frank A. Chervenak, MD, and Laurence B. McCullough, PhD
New York, New York, and Houston, Texas

We defend the view that the obstetrician's response to the abortion controversy cannot be based on accounts of the independent moral status of the fetus, because all such accounts are irresolvably disputable. That response, however, can be based on an account of the dependent moral status of the fetus. For such an account the central question is, "When is the fetus a patient?" Viable fetuses are patients. Nonviable third-trimester fetuses are not patients. Previable fetuses are patients solely as a function of the woman's autonomous decision to confer such status. The abortion of a viable fetus is, with few exceptions, never ethically justified, because it is a patient. The abortion of nonviable third-trimester fetuses (i.e., premature termination of pregnancy) is justified when the pregnant woman consents to it. Abortion of the previable fetus is justifiable when the pregnant woman consents to it. We distinguish between professional and private conscience. We identify the limits of the former and the legitimate role of the latter, especially in the area of religious beliefs. Finally, we address the implications of our view for residency training programs. The most important of these implications is that requiring all residents to perform abortions is ethically unjustifiable. (Am J Obstet Gynecol 1990;163:1425-9.)

Key words: Abortion, ethics, viability, residency education

Abortion ranks as one of the most divisive issues affecting contemporary American society. This divisiveness has the potential to extend to the obstetric profession because obstetricians counsel pregnant women and perform abortions. The voluminous literature on the ethics of abortion can be accurately described as a controversy for which closure on a common ground is impossible.[1] This is because of the markedly varied arguments about when, why, and by whom there might be ethical obligations owed to the fetus. As a consequence, it seems fair to ask, Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? We believe that the answer is yes.

We understand obstetric ethics to be a subspecialty of the field of medical ethics. Medical ethics seeks to identify, on the basis of ethical principles, the ethical obligations of physicians to patients and to third parties to the patient-physician relationship, e.g., family members, hospitals, and public and private payers.[2, 3] Obstetric ethics seeks to identify the ethical obligations of the physician to the pregnant patient and to third parties to the patient-physician relationship. Of special interest is the issue of whether the physician has ethical obligations to the fetus. The history of medical ethics in the West began with Hippocrates.[4] Obstetric ethics is a part of this history and continues to be developed in the medical and philosophical literature. These discussions embrace the full range of ethical issues in obstetric practices, e.g., maternal-fetal conflict,[5] fetal diagnosis and treatment,[6] and abortion.[7]

Theological and philosophical responses

The first response of many persons to the abortion controversy is to try to establish theological or philosophical grounds for the independent moral status or lack of independent moral status of the fetus.[1, 8-10] Independent moral status would mean that the fetus in and of itself generated its own moral rights, which would have to be acknowledged and respected by everyone. The disabling problem with these responses is that they are so varied that no common ground has emerged and clinical application therefore has been impossible. Theological traditions are marked by deep disagreement, both intramurally and transmurally. In these matters, moreover, theological traditions have been unable to agree on a single perspective from which their disputes can be universally and authoritatively settled. Philosophical traditions have fared no better. Indeed, the voluminous philosophical literature on the ethics of abortion is markedly heterogeneous in its methods and results. Thus no single philosophical perspective can claim with legitimacy a primacy over any other. In 1973, the United States Supreme Court stated that philosophers and theologians have been unable to settle

From the Divisions of Ultrasound and Ethics, Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center, and the Departments of Medicine and Community Medicine, Center for Ethics, Medicine, and Public Issues, Baylor College of Medicine. Reprint requests: Frank A. Chervenak, MD, The New York Hospital–Cornell Medical Center, 525 East 68th St.–M713, New York, NY 10021.
6/1/22484

the question of when human life begins, i.e., when the fetus has independent moral status. There has been no progress in the intervening years. Indeed there seems to be no common ground available.[7]

The implication for obstetricians of this lack of progress is that they should be deeply suspicious of any responses to the abortion controversy that assert an indisputable account about the independent moral status or lack of independent moral status of the fetus. Suspicion is justified because no such account can claim final intellectual or moral authority, given the necessarily disputable nature of all accounts of the independent moral status of the fetus.

The response of obstetric ethics

Obstetric ethics need not be paralyzed, however, because it does not need an account of the *independent* moral status of the fetus. This is because obstetric ethics asks a different question: When is the fetus a patient? This question is not about the independent moral status of the fetus. Instead it is a question about the *dependent* moral status of the fetus; that is, the moral status of the fetus is a function of whether the fetus always has the moral status of being a patient. It is not a function of some intrinsic property of the fetus, e.g., being a human being or being unique genetically. Obstetric ethics is competent to address the question of whether the fetus is always a patient.

We shall argue the following: When the fetus is not a patient, the response of obstetricians to the abortion controversy is clear. Pregnant women in consultation with their obstetricians should be permitted to make their own decisions for or against the moral status of the fetus and therefore about abortion. However, when the fetus is a patient, the response of obstetric ethics to the abortion controversy is that this freedom sometimes can be justifiably limited.

The distinctive advantage of obstetric ethics is that it can answer the question, "When is the fetus a patient?" on secular, philosophical grounds. Theological sectarian grounds cannot be invoked at this stage of inquiry because obstetric ethics is secular in character for two reasons. First, medicine as a social institution rests on secular intellectual foundations, the biomedical sciences, and a secular social sanction, licensure by the state. These are factual matters about the development of medicine in America. Second, as Engelhardt[11] has convincingly argued, (1) only a secular approach to medical ethics (of which obstetric ethics is a subspecialty) is adequate in a pluralistic society and (2) only philosophical methods are capable of articulating secular medical ethics in a pluralistic society. No theological approach can satisfy these two criteria.

Accounts of the independent moral status of the fetus are all based on claims about the *beginning* of fetal development, e.g., the "moment" of conception, or when twinning becomes impossible.[1,7] Because such accounts are all disputable, we anchor the continuum of ethical obligations to the fetus as a patient at the *end* of the continuum of human development. This is when independent moral status is not disputable, namely, when human beings become persons. Persons, in the philosophical (not legal) sense of the word, are rational, self-conscious entities that are ends in themselves. As such, they independently generate their own moral status in an unambiguous fashion. Such moral status also generates a person's rights and autonomy-based obligations to other persons. The biologically and socially sufficient conditions for becoming a person probably occur sometime during the latter half of, or shortly after, the neonatal period. Engelhardt's is perhaps the most thorough exposition of the implications of respect for persons for bioethics.[11] However, that persons generate their own independent moral status is a commonly accepted view in ethical theory.

Persons can become patients when they consent to do so. Pregnant women can become patients, therefore, when they consent to do so. However, one need not be a person to be a patient, as is plainly the case in neonatal medicine. This is because in medical ethics the physician can have beneficence-based obligations to human beings independently of whether they are persons. These obligations derive from the physician's role as a care giver and exist when it is reasonable to seek those human goods that medicine as a social institution is competent to achieve. These goods are the prevention of premature or unnecessary death and the prevention, cure, or amelioration of disease, injury, handicap, and unnecessary pain and suffering. Medicine's competence in this respect is a function of biomedical knowledge, clinical skills, and technical capacity.[3]

This line of reasoning applies to the question, "When is the fetus a patient?" in the following way. We understand the viability of a fetus to mean that it can survive delivery into the neonatal period and infancy, given appropriate biologic potential and the availability of biomedical interventions applied under the test of reasonableness, explained below. Viability thus has ethical significance because the viable fetus can become a person. Because viability involves both biologic potential and technologic capacity,[12] it is not an intrinsic property of the fetus but acquires its ethical significance in terms of the end point of the continuum of development into a person. Viability thus concerns the dependent moral status of the fetus. Moreover, viability will depend on the expertise of the medical community caring for a particular patient, on inherent variability of fetal maturation, and intrinsic uncertainties in the

determination of fetal gestational age. Hence it is not possible to establish a universal cutoff date for gestational age.

In medical ethics there is a beneficence-based obligation to intervene to prevent the death of a human being when it is possible and reasonable to do so. It is possible to do so when the human being in question is accessible to the physician for the purposes of applying clinical interventions. It is reasonable to do so when the attempt to intervene to prevent death does not involve overly burdensome risks of morbidity and mortality and is expected to result in net benefit.

The viability of the fetus means that it is accessible to the physician to become a patient, as a function of biomedical knowledge, clinical skills, and technologic capacity. Hence the physician has a beneficience-based obligation to intervene on behalf of the viable fetus, when it is reasonable to do so.[13]

There are two limits on this obligation. The first involves obligations owed to the pregnant woman.[13] The second involves the likelihood that the end point of the continuum of human development can be achieved; that is, the ethical implications of viability for the weight of the physician's and the pregnant woman's actual beneficence-based obligations to the fetus as a patient in particular cases are variable.

For some fetuses there is the high probability of diagnosis of an anomaly that will result with a very high probability in death as an outcome or survival with a very high probability of irreversible and severe cognitive developmental deficit. The beneficence-based obligations to intervene to prevent death for such fetuses are minimal. Minimal beneficence-based obligations are consistent with nonaggressive management of the pregnancy but not its termination, because direct killing violates minimal beneficence-based obligations to prevent the fetus' death because the woman has implicitly accepted such obligations by taking her pregnancy to viability.[14, 15]

Some fetuses have a prognosis of expected survival with no, or manageable, deficit of cognitive developmental capacity. Beneficence-based obligations to such fetuses are more than minimal. The test for overriding more than minimal beneficence-based obligations to the fetus is more demanding, especially when there is no maternal indication for not completing the pregnancy.[14] The justification for this view is that abortion does not prevent significant health risks to the pregnant woman and, by taking her pregnancy beyond viability, she has implicitly accepted an obligation to accept the normal risks of childbirth.[14] Thus there are circumstances when ethical obligations to the fetus as a patient can legitimately take precedence over the woman's intentions regarding her pregnancy.

This precedence is not absolute, however, because there may be reasonable exceptions. This is because pregnancy can very rarely pose unreasonable risks to the woman's life or health after fetal viability, risks that she is not ethically obligated to accept.[13] Hence she should be free to determine the disposition of her pregnancy when it poses significant risks of serious morbidity (e.g., stroke or blindness) or mortality to her. In such cases, if the woman were to decide to terminate the pregnancy, every effort should be made to minimize fetal morbidity and mortality, because the fetus remains a patient.

Some third-trimester fetuses are not viable because of lethal anomalies; they are therefore not patients, and no beneficence-based obligations are owed to them to intervene to prevent an inevitable death. This is the case when the diagnosis is certain and when there is certainty of death as the outcome of the anomaly diagnosed or, in cases of short-term survival, certainty of the absence of cognitive developmental capacity.[14, 16] Abortion of nonviable third-trimester fetuses (i.e., premature termination of pregnancy) is ethically permissible because there are no beneficence-based obligations to the fetus to prevent death on the part of the pregnant woman or physician.[14] Abortion of such fetuses is justified therefore when the pregnant woman consents to it.

No link can be established between the previable fetus and the end point of the continuum of human development via biomedical technologic capacity. This is simply what *previable* means. The only possible remaining link is the pregnant woman's choice about the disposition of her pregnancy. However, because that link is solely a function of her autonomous choice, the pregnant woman is under no ethical obligation to regard the previable fetus as a patient. The physician is therefore under no such obligation for the same reason. Thus, before viability, a pregnant woman is free to withhold, confer, or, having once conferred, withdraw the status of being a patient from the fetus. In other words, for secular obstetric ethics, the abortion controversy regarding previable fetuses is resolved for physicians by the autonomous decision of the woman regarding her pregnancy and the dependent moral status of the fetus as a patient.

There is in the literature on obstetric ethics an approach that holds that all of obstetric ethics is essentially a function of the pregnant woman's autonomy.[5, 17] On this approach, the distinction among viable, nonviable, and previable fetuses would be ethically insignificant. From this it would follow that the pregnant woman is free to withhold, confer, or, once having conferred, withdraw the status of being a patient from the fetus throughout pregnancy. With respect to previable and

nonviable fetuses, therefore, this approach to obstetric ethics and ours are in agreement on the response of obstetric ethics to the abortion controversy.

Some might object at this point that physicians have an obligation not to be an agent of harm to the previable fetus, independently of what a pregnant woman may choose; that is, the principle of *primum non nocere,* or nonmaleficence,[2] may be violated. We have two responses to this objection. First, *primum non nocere* is neither an absolute nor, indeed, a first principle of medical ethics in general or of obstetric ethics in particular. Instead, nonmaleficence is secondary to beneficence.[3] Second, even if *primum non nocere* were admitted as a primary consideration, it would only show that a physician should not harm a patient. It cannot be invoked to show that a biologic human being *is* a patient. The principle of beneficence cannot be used for this purpose either. Human beings become patients when they become accessible to physicians for the purposes of applying reasonable clinical interventions, which is not the case for previable or nonviable fetuses.

In summary, the response of obstetric ethics to the abortion controversy is the following. The abortion of previable fetuses, the central focus of the abortion controversy because virtually all abortions are performed before viability, involves no violation of the principles of secular obstetric ethics. Neither does the abortion of nonviable third-trimester fetuses, those with lethal anomalies. In both cases abortion does not involve violation of beneficence-based obligations. *Primum non nocere* is not violated either. In our view the abortion of viable fetuses to which minimal and more than minimal beneficence-based obligations are owed is not ethically justifiable, with the exceptions of the woman's decision to give precedence to her own life or to prevent significant health risks she is not obligated to accept.[14]

The abortion controversy and the distinction between professional and private conscience

Secular obstetric ethics generates the ethical obligations that an obstetrician as a health care professional has to patients. Secular obstetric ethics transcends differences of private morality because it binds the conduct of all obstetricians, as obstetricians. Obstetricians, however, also have private consciences, which are shaped by the private morality of each individual. Private morality is a function of such individual factors as personal experience, family upbringing, and religious tradition. In contrast to professional conscience, private conscience is variable because of the striking hetereogeneity of the sources of private conscience. Professional conscience governs the response to the abortion controversy of the obstetrician as a professional person bound by the obligations of a professional role. Private conscience cannot bear on the professional role but governs only the obstetrician's responses in his or her nonmedical roles of lay person and private citizen.

Professional conscience governs the obstetrician's obligations to his or her patient. Private conscience governs whether continuing to serve as an obstetrician to a particular patient obligates the physician to act in such a way as to produce intolerable burdens on his or her private moral convictions, values, and beliefs, including those of theological origin. Respect for the integrity of the private conscience of each individual obstetrician means that some human beings justifiably cannot become, or can cease for particular purposes to be, patients of a particular obstetrician. Thus, asserting respect for autonomy in the form of respect for the moral convictions, including religious convictions, of private conscience can sometimes be a legitimate ethical claim on the part of the obstetricians; that is, secular obstetric ethics underscores the legitimate role of religious belief in the formation of the private conscience of the obstetrician.

There are important limits on such a claim. Matters of private conscience do not govern the physician's response to the abortion controversy in obstetric ethics, only the morality of abortion in one's private moral life, i.e., in terms of one's response to one's roles other than that of being an obstetrician. Thus, on the basis of private conscience, one has no intellectual license to judge the morality of pregnant women who contemplate termination or continuation of their pregnancies or to judge adversely behavior of colleagues that is consistent with secular obstetric ethics as explained in the preceding section. Private conscience justifies only withdrawing from particular cases. When withdrawal is undertaken, professional conscience requires that the physician see to it that the pregnant woman's care is transferred in an orderly and safe manner to a colleague whose private conscience is not violated by the pregnant woman's decisions. No judgments about the morality of those decisions should be expressed to the pregnant woman, for whom the obstetrician acts exclusively in the role of physician, not a private person.

While private-conscience-based moral objections to a pregnant woman's decision should never be used to judge her, it does not follow that physicians as citizens have no freedom to add their private-conscience-based views on the morality of abortion to the ongoing public debates about abortion and public policy in our country. In doing so, however, when they invoke their professional mantle, their contribution must be governed in its moral content by secular obstetric ethics. Otherwise, obstetricians can join the political fray as interested lay persons or citizens, with no special or authoritative perspective *as obstetricians* on the abortion controversy. When obstetricians contribute as obstetricians to public debate on any topic, they are constrained by secular

medical ethics and its response to the question, "When is the fetus a patient?"

Implications for obstetric residency programs

The preceding has important implications for obstetric residency programs. We, like many others, have heard anecdotal reports that applicants to some residency programs sometimes learn, in one way or another, that all residents in those programs are required to perform abortions. We have inquired of the American College of Obstetricians and Gynecologists and the Council on Residency Education in Obstetrics and Gynecology for data on this subject, and we were told that no data have been collected.

Such an educational policy raises troubling ethical issues because it denies the integrity of private conscience and misinterprets secular obstetric ethics and its implications for professional conscience. As to the latter, all that obstetric ethics can show is that, when the fetus is not a patient, a pregnant woman possesses unrestricted freedom regarding the disposition of the pregnancy. Obstetric ethics does not show therefore that every obstetrician should carry out a pregnant woman's request for an abortion, because doing so may violate private conscience in some cases. Any educational program that requires all residents to perform abortions rests on a misunderstanding of the limits of professional conscience. That such a policy could unjustifiably violate private conscience is therefore plain.

This is different from the case that, on the basis of the religious traditions of the sponsoring institution, a residency program prohibits residents from being involved in performing abortions at that institution. This is because there is a well-established tradition in our society that religious institutions have the right to protect the integrity of their traditions so long as no harm befalls innocent, nonconsenting human beings. Because such a policy does not tell residents what to believe as a matter of private conscience, because such a policy is consistent with the limits of obstetric ethics, and when such a policy does not eliminate access to abortion in a community, it is ethically justified.

We are fully cognizant of the possibility of abuse of a policy that permits exceptions on the grounds of private conscience. The best remedy to prevent such abuse is (1) to require residents to document the basis of their objections to performing abortions in a rigorous way, analogous to the requirement imposed on citizens who seek conscientious objector status regarding military service, and (2) to define clearly the additional duties expected of residents in lieu of not performing abortions.

Comment

The abortion controversy in our country will not disappear. It seems to intensify with each new United States Supreme Court ruling and actions of state legislatures and governors on the subject. Obstetric ethics, precisely because of its secular character, equips physicians to respond to this ongoing controversy in the disciplined, reasoned fashion expected of members of a learned profession. By submitting to the intellectual discipline of secular obstetric ethics, physicians can act to prevent the divisiveness of the abortion controversy from extending to the medical profession.

REFERENCES

1. Curran CE. Contemporary debate in philosophical and religious ethics. In: Reich W, ed. Encyclopedia of bioethics. New York: Macmillan 1978:17-26.
2. Beauchamp TL, Childress JF. Principles of biomedical ethics. 3rd ed. New York: Oxford University Press, 1989.
3. Beauchamp TL, McCullough LB. Medical ethics: the moral responsibilities of physicians. Englewood Cliffs, New Jersey: Prentice-Hall, 1984:22-51.
4. Reich WT, ed. History of medical ethics. In: Encyclopedia of Bioethics. New York: Macmillan, 1978:876-1007.
5. Elias S, Annas GJ. Reproductive genetics and the law. Chicago: Year Book, 1987.
6. Evans MI, Fletcher JC, Dixler AO, Schulman JD. Fetal diagnosis and therapy. In: Science, ethics, and the law. Philadelphia: JB Lippincott, 1989.
7. Callahan S, Callahan D, eds: Abortion: understanding differences. New York: Plenum Press, 1984.
8. Feldman DM. Abortion: Jewish perspectives. In: Reich WT, ed. Encyclopedia of Bioethics. New York: Macmillan, 1978:5-9.
9. Connery JR. Abortion: Roman Catholic perspectives. In: Reich WT, ed. Encyclopedia of Bioethics. New York: Macmillan, 1978:9-13.
10. Nelson JB. Abortion: Protestant perspectives. In: Reich WT, ed. Eycyclopedia of Bioethics. New York: Macmillan, 1978:13-17.
11. Engelhardt HT Jr. The foundations of bioethics. New York: Oxford University Press, 1986.
12. Mahowald M. Beyond abortion: refusal of cesarean section. Bioethics 1989;3:106-21.
13. Chervenak FA, McCullough LB. Perinatal ethics: a practical method of analysis of obligations to mother and fetus. Obstet Gynecol 1985;66:442-6.
14. Chervenak FA, McCullough LB. An ethically justified, clinically comprehensive management strategy for third-trimester pregnancies complicated by fetal anomalies. Obstet Gynecol 1990;73:311-6.
15. Chervenak FA, McCullough LB. Nonaggressive obstetric management: an option for some fetal anomalies during the third trimester. JAMA 1989;261:3439-40.
16. Chervenak FA, Farley MA, Walters L, et al. When is termination of pregnancy during the third trimester morally justifiable? N Engl J Med 1984;310:501-4.
17. Annas GJ. Protecting the liberty of pregnant patients. N Engl J Med 1987;316:1213-4.

Interleukin-1β, human leukocyte antigen HLA-DRα, and transforming growth factor-β expression in endometrium, placenta, and placental membranes

Scott Kauma, MD, Dennis Matt, PhD, Stephen Strom, PhD, David Eierman, PhD, and Thomas Turner, BS

Richmond, Virginia

Maternal immune recognition of the fetal semiallograft appears to be necessary and beneficial for fetal survival and growth. Interleukin-1β and human leukocyte antigen HLA-DR are important for foreign antigen recognition by the immune system, whereas transforming growth factor-β inhibits many of the immunostimulatory properties of interleukin-1β. In this study we found that first-trimester decidua and term placental membranes expressed significantly higher levels of interleukin-1β, interleukin-1β messenger ribonucleic acid, and human leukocyte antigen HLA-DRα messenger ribonucleic acid than proliferative and secretory endometrium. Fetal placenta had little, if any, interleukin-1β, interleukin-1β messenger ribonucleic acid, or human leukocyte antigen HLA-DRα messenger ribonucleic acid expression. All tissues found at the maternal-fetal interface, including first-trimester decidua, placenta, and placental membranes, contained transforming growth factor-β and expressed transforming growth factor-β, messenger ribonucleic acid. On the basis of these findings, we suggest that the increase in decidual interleukin-1β and human leukocyte antigen HLA-DRα during pregnancy may be involved in maternal recognition of the fetal semiallograft and that transforming growth factor-β production may regulate the local maternal immune response and prevent rejection of the fetus. (Am J Obstet Gynecol 1990;163:1430-7.)

Key words: Interleukin-1β, human leukocyte antigen HLA-DRα, transforming growth factor-β, endometrium, placenta

Maternal immunologic recognition of the developing fetal semiallograft is a normal physiologic response during pregnancy and may be important for fetal survival.[1] Several studies have concluded that certain cases of habitual miscarriage are a result of inadequate maternal immune recognition of a fetus whose paternal human leukocyte antigen complement too closely resembles that of the mother.[2,3] Maternal decidua, which is in direct contact with fetal trophoblast, contains a relatively large number of macrophages and lymphocytes.[4] These cell types are capable of complex immunologic interactions, including allograft recognition, which is mediated in part by various cytokines and the major histocompatibility cell surface antigens.[5] Consequently, the regulation of cytokine production and major histocompatibility antigen expression at the maternal-fetal interface may play an important role in maternal immunologic recognition of the fetus and its subsequent survival.

Interleukin-1 (IL-1) is an immunoregulatory protein whose biologic properties include stimulation of T-cell cytokine production, T- and B-cell proliferation, and foreign antigen recognition.[6] There are two forms of IL-1, IL-1α and IL-1β, which have identical biologic activities but are distinctly separate peptides encoded by separate genes. Although the in vivo expression of IL-1 has not been demonstrated for normal endometrium and decidua, it has been reported that term decidua in vitro can produce IL-1α and, to a lesser extent, IL-1β in response to bacterial endotoxin.[7]

HLA-DR is a major histocompatibility type II cell

From the Department of Obstetrics and Gynecology, Medical College of Virginia, Virginia Commonwealth University.
Supported by a grant from the A. D. Williams Foundations, Medical College of Virginia, Virginia Commonwealth University, and by grant No. BC579 from the American Cancer Society.
Received for publication March 21, 1990; revised July 2, 1990; accepted July 17, 1990.
Reprint requests: Scott W. Kauma, MD, Medical College of Virginia, Department of Obstetrics and Gynecology, Box 34, MCV Station, Richmond, VA 23298.
6/1/23861

surface antigen found mainly on activated lymphocytes and cells of monocyte-macrophage lineage. Antigen-presenting cells, many of which are tissue macrophages, couple foreign antigens to HLA-DR and then externalize the immune complex to the cytoplasmic membrane where it is recognized by T and B lymphocytes.[5] The presence of HLA-DR–bearing cells in decidua has been demonstrated by immunohistochemistry.[4] Both HLA-DR and IL-1 expression are important for effective foreign antigen recognition and subsequent lymphocyte activation and proliferation.[5]

Although some degree of maternal immune recognition of the fetus occurs during pregnancy, regulation of the local maternal immune response may be necessary to prevent immune rejection of the fetus. Transforming growth factor-β (TGF-β) is a multifunctional peptide with potent immunosuppresive activities including inhibition of IL-1β–induced lymphocyte proliferation,[8] lymphokine-activated killer cell activity,[9] cytotoxic T-cell activity, natural killer cell activity,[10] and macrophage cytotoxicity.[11] Although both TGF-β[12] and TGF-β[13] messenger ribonucleic acid (mRNA) have been isolated from term placenta, the role of TGF-β in the immunoregulation of the maternal-fetal allograft is largely unexplored.

Because IL-1β, HLA-DR and TGF-β are important immunoregulatory peptides that may play a role in the local maternal immune response to the fetus, this study was designed to characterize the expression of these peptides in tissues found at the maternal-fetal interface during the course of the normal menstrual cycle and during pregnancy.

Material and methods

Tissue samples for this study were obtained from women undergoing elective pregnancy termination by curettage (n = 17), from women with normal cycles undergoing hysterectomy for benign conditions (leiomyomas, nine cases; endometriosis, three, pelvic pain or endometritis, one), and from nonlaboring, uncomplicated repeat term cesarean sections (n = 5). Written consent from the patient and approval by the Institutional Committee on the Right of Human Subjects were obtained before collection of any tissue samples for this study. Placenta and decidualized endometrium from pregnancy terminations were suspended in normal saline solution, washed free of blood, separated under low-power magnification, and frozen in liquid nitrogen within 10 minutes of surgical removal and then stored at −80° C. Endometrium from hysterectomy specimens was scraped from the uterine wall with a scalpel. Within 15 minutes of delivery, term placental membranes and term placental villi dissected free from the decidua basalis were washed in normal saline solution to remove contaminating blood. These tissues were immediately frozen in liquid nitrogen and stored at −80° C. A small portion of the endometrial sample from each hysterectomy specimen was formalin fixed and paraffin embedded; thin sections were stained with hematoxylin and eosin for dating according to the method of Noyes et al.[14] Pregnancy dating was based on a reliable date of the last menstrual period combined with uterine size, ultrasonographic examination, or both of the latter methods.

Northern blot analysis of IL-1β, HLA-DRα, and TGF-β_1. Total ribonucleic acid (RNA) was isolated from the samples by acid guanidinium thiocyanate-phenol-chloroform extraction according to the method of Chomczynski et al.[15] with the following modification. After the final total RNA pellet was dissolved in diethylprocarbonate–treated water, the RNA was further purified by phenol-chloroform extraction followed by chloroform extraction. The RNA was then precipitated with ethanol and pelleted by centrifugation. The final RNA pellet was dissolved in diethylprocarbonate–treated water and the total RNA concentration determined by absorption spectrophotometry at 260 nm.

Total RNA (30 μg) was fractionated by electrophoresis in a denaturing formaldehyde 1% agarose gel in MOPS buffer (0.04 mol/L morpholinopropanesulfonic acid, 10 mmol/L sodium acetate, 1 mmol/L ethylenediaminetetraacetic acid, pH 7.0). The integrity and identical quantity of each RNA sample was ensured by staining the gel with ethidium bromide and visualizing the gel under ultraviolet transillumination. The RNA was transferred to nitrocellulose by capillary diffusion with 20x saline–sodium citrate buffer (SSC) (3 mol/L sodium chloride, 0.3 mol/L sodium citrate, pH 7.0) and then baked at 80° C under vacuum for 2 hours. Prehybridization of the filter was performed with hybridization solution (50% formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% sodium dodecyl sulfate, 0.1 mol/L sodium phosphate, 100 μg/ml sheared salmon sperm DNA, 0.75 mol/L sodium chloride, 0.75 mmol/L sodium citrate, pH 7.0) for 1 hour at 42° C. The filters were hybridized with 1×10^6 cpm/ml denatured phosphate 32 deoxycytidine triphosphate (^{32}P-dCTP)–labeled IL-1β or HLA-DRα, complementary DNA probes in hybridization solution at 42° C for 16 to 24 hours. Posthybridization washes were performed in 4x SSC, and finally 0.1x SSC (containing 0.02 mol/L sodium phosphate, 0.1% sodium dodecyl sulfate, pH 6.4) for 30 minutes each at 56° C. TGF-β was similarly hybridized and washed except that the hybridization temperature was 56° C and the posthybridization wash temperature was 65° C. The nitrocellulose filters were then exposed to Kodak XAR radiographic film (Eastman Kodak, Rochester, N.Y.) with intensifying screens at −80° C.

A 1.4 kb *Eco*RI cut complementary DNA insert specific for human interleukin-1β (S. Haskill, PhD, De-

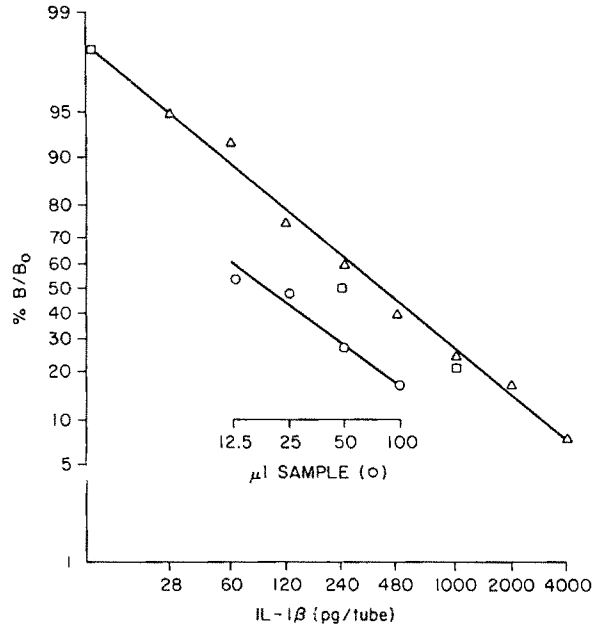


Fig. 1. The logit-log graph of the standard curve for IL-1β (△). To validate the assay, a placental membrane sample was serially diluted (○) in a separate set of experiments and 240 or 1000 pg of standard IL-1β was added to a placental sample (□) and compared with the standard curve.

partment of Obstetrics and Gynecology, University of North Carolina, Chapel Hill), a 3.1 kb *Eco*RI cut genomic clone specific for HLA-DR1 (S. Weissman, MD, Department of Human Genetics, Yale University, New Haven, Conn.) and a 1.05 kb *Eco*RI cut insert specific for human TGF-β_1 (R. Derysnck, PhD, Genentech, Inc., San Francisco) were used as probes in this study. These specific complementary DNA inserts were radiolabeled with ^{32}P-dCTP by random prime oligonucleotide labeling (Pharmacia Oligo Labeling kit, Pharmacia LKB Biotechnologies, Inc., Piscataway, N.J.). The relative expression of IL-1β, HLA-DRα, and TGF-β_1 mRNA was determined by densitometric analysis of the autoradiograms. Statistical analysis was performed with analysis of variance.

IL-1β radioimmunoassay (RIA). To measure IL-1β protein in endometrium, placenta, and placental membranes, 250 mg of tissue was homogenized in 1 ml of phosphate-buffered saline solution pH 7.4 that contained 0.8% 3-[(3-cholamidopropyl)dimethylammonio]1-propanesulfonate, 10 mmol/L phenylmethylsulfonyl fluoride, and 1 mmol/L leupeptin (extract buffer). The samples were centrifuged at 12,000 *g* for 45 minutes at 4° C and the supernatant recovered for IL-1β and total protein determination. Total protein concentration in the supernate was determined in duplicate by means of a standard protein assay (Bio-Rad protein assay, Bio-Rad Laboratories, Richmond, Calif.). IL-1β concentration was determined in the supernate by RIA and expressed as picograms of IL-1β per milligram of total protein. All samples that contained less than the detectable amount of IL-1β in the assay (50 *pg*/100 μl) were assigned the minimal detectable value of 50 *pg*/100 μl sample, and expressed as picograms of IL-1β per milligram of total protein.

The IL-1β RIA used in this study was developed with commercially available reagents. The samples (100 μl) in duplicate and human recombinant IL-1β standards (R and D Systems, Inc., Minneapolis) were incubated with 0.35 μg/ml rabbit antihuman IL-1β IgG (R and D Systems) for 24 hours at 4° C in a total volume of 400 μl of phosphate-buffered saline solution with 0.5% bovine serum albumin, pH 7.4. The samples were then incubated with 100 μl (10,000 cpm, 1000 Ci/mmol) of the iodine 125–labeled human recombinant IL-1β (Amersham Corp., Arlington Heights, Ill.) for an additional 24 hours at 4° C. The samples were then incubated with 100 μl of a 1 : 10 dilution of antirabbit IgG agarose in phosphate-buffered saline solution (Sigma Chemical Company, St. Louis) for 2 hours at room temperature with constant shaking. The bound counts were pelleted by centrifugation with the addition of 1 ml of a 0.125% solution of crystobalite (Manvill Company, Denver) to help maintain pellet integrity. The supernatant was removed and each pellet was counted in a γ-counter. To validate the assay, samples that were either serially diluted or to which known amounts of the IL-1β standard were added were compared with the standard curve to demonstrate appropriate parallelism (Fig. 1). The intraassay coefficient of variability was <10%, and all samples of this study were run in the same assay to eliminate interassay variability. Statistical analysis was performed with analysis of variance.

Characterization of tissue-extractable TGF-β. TGF-β–like bioactivity was extracted from first-trimester gestational endometrium, first-trimester placenta, term placenta, and term placental membranes as previously described.[16] Briefly, 10 gm of tissue was homogenized in concentrated hydrochloric acid/95% ethanol (1 : 50) and then incubated at 4° C for 24 hours. The homogenate was centrifuged and the supernate was then recovered and precipitated overnight at 4° C with the addition of ethanol and ethyl ether. The resulting precipitate was dissolved in 1 mol/L acetic acid and clarified by centrifugation; the supernate was then lyophilized. The lyophilized extract was dissolved in 2 ml of 1 mol/L acetic acid and applied to a 36 × 2.5 cm Bio Gel P-60 column (Bio-Rad Laboratories, Richmond, Calif.) equilibrated in 1 mol/L acetic acid. The column was eluted with a 1 mol/L acetic acid at 12 ml/hour collecting 3 ml fractions. A portion (500 μl) of each

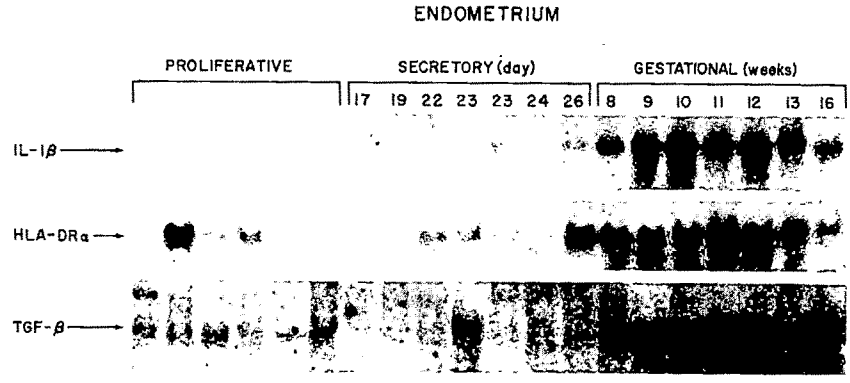


Fig. 2. Northern blot of proliferative, secretory, and decidualized gestational endometrium for IL-1β, HLA-DRα, and TGF-β_1.

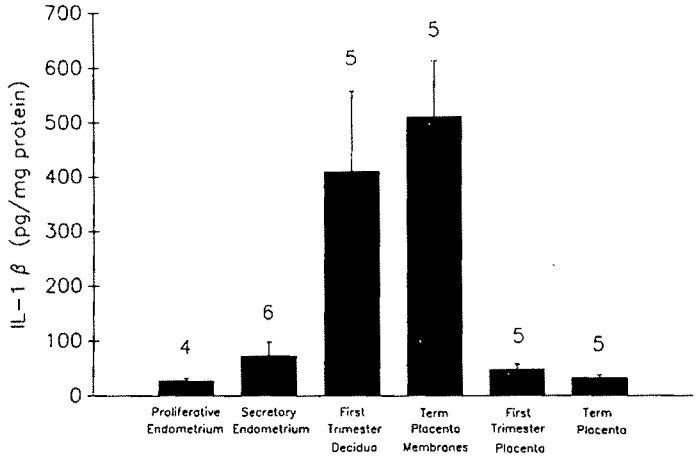


Fig. 3. First-trimester decidua and term placental membranes contain significantly more extractable immunoreactive IL-1β protein ($p < 0.004$) than proliferative endometrium (nondetectable samples, four) and secretory endometrium (nondetectable samples, one), first-trimester placenta (nondetectable samples, five), and term placenta (nondetectable samples, five). *Closed bars* represent the mean levels ± SEM of IL-1β for each group.

column fraction in duplicate was then lyophilized and finally dissolved in 50 μl of 0.01 mol/L hydrochloric acid and used for the TGF-β assay. The tissue extract column fractions were examined for the presence of TGF-β–like bioactivity by the anchorage independent growth assay of normal rat kidney cells (clone 49F).[17] The normal rat kidney cell growth conditions consisted of two layers of media containing agar in 35 × 10 mm culture dishes (Falcon, Becton-Dickinson, Cockeysville, Md.). The bottom layer consisted of 0.6% Bacto-agar (Difco Laboratories, Detroit), 5% fetal calf serum (Gibco, Grand Island, N.Y.), 4 ng/ml epidermal growth factor (Collaborative Research, Inc., Bedford, Mass.), and 50 μg/ml gentamicin sulfate (Sigma) in 1 ml Dulbecco's modification of Eagle medium (Gibco). The top layer was identical to the bottom layer except for a lower Bacto-agar concentration of 0.4% and the addition of 3×10^3 normal rat kidney cells and 50 μl of the fractionated tissue extracts of TGF-β standards (R and D Systems). The plates were then incubated at 37° C in a humidified 95% air/5% carbon dioxide incubator. After 14 days of growth colonies 100 μm or larger were counted and scored. The number of colonies that grew in the fraction sample group were compared with the number of colonies that grew with use of the porcine TGF-β standards and were reported as TGF-β equivalents per fraction.

Results

The IL-1β complementary DNA probe hybridized with a 1.8 kb mRNA species found in decidualized endometrium and term placental membranes. IL-1β mRNA expression was found to be increased twenty-fold ($p < 0.006$) in a decidualized endometrium from

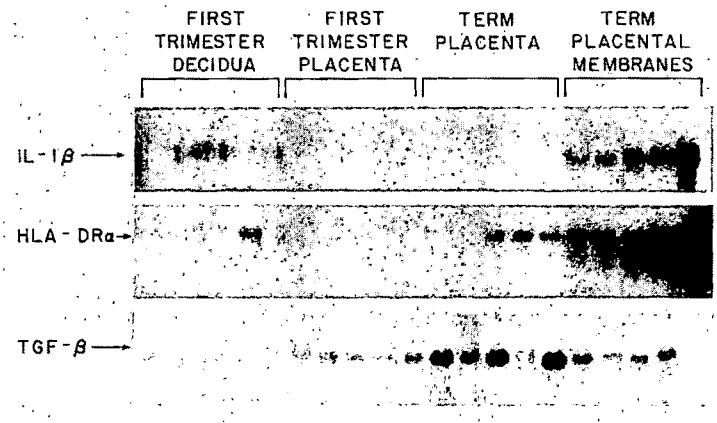


Fig. 4. Northern blot of first-trimester decidua, first-trimester placenta, term placenta, and term placental membranes for IL-1β, HLA-DRα, and TGF-β_1.

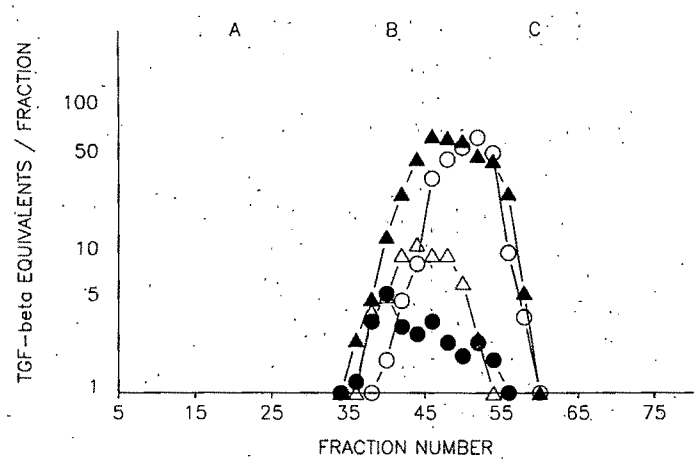


Fig. 5. TGF-β bioactivity found in Bio-gel P-60 column fractions of acid-ethanol extractable protein from decidua (●), first-trimester placenta (△), term placental membranes (○), and term placenta (▲). The column was calibrated with bovine serum albumin (60,000 molecular weight) *(A)*, chymotrypsinogen (27,000 molecular weight) *(B)*, and lysozyme (14,000 molecular weight) *(C)*. One TGF-β equivalent is equal to 1 ng of porcine platelet TGF-β standard.

first-trimester pregnancies when compared with endometrium from the normal menstrual cycle (Fig. 2). Except for a single proliferative endometrial sample that had histologic evidence of endometritis, IL-1β mRNA expression first appeared in secretory endometrium beginning on cycle day 23. All later secretory and gestational decidualized endometrial samples demonstrated IL-1β mRNA expression. Tissue-extractable IL-1β in decidualized gestational endometrium (410 pg/mg total protein) was 7.5-fold ($p < 0.004$) greater than in proliferative endometrium and secretory endometrium (Fig. 3). Term placental membranes, which contain fetal amnion, fetal cytotrophoblast (chorion), and maternal decidualized endometrium, had slightly higher levels of tissue-extractable IL-1β (510 pg/mg total protein) and significantly higher (sevenfold, $p < 0.001$) expression of IL-1β mRNA when compared with first-trimester gestational endometrium (Figs. 3 and 4). Although maternal gestational decidualized endometrium expressed high levels of IL-1β, fetal first-trimester and term placenta expressed little, if any, IL-1β protein or IL-1β mRNA (Figs. 3 and 4).

The HLA-DRα probe hybridized with a 1.6 kb mRNA species found in both maternal and fetal tissues. The relative change in expression of HLA-DRα in endometrium was similar to IL-1β (Fig. 2). HLA-DRα mRNA expression was increased threefold ($p < 0.02$) in gestational endometrium when compared with proliferative and secretory endometrium. However, unlike IL-1β mRNA expression in endometrium, HLA-DRα was also expressed at low levels in most proliferative and secretory endometrial samples. Term placental

membranes expressed slightly higher levels of HLA-DRα mRNA when compared with gestational decidualized endometrium and term placenta, whereas first-trimester placenta had little, if any, HLA-DRα expression (Fig. 4).

Because immune rejection of an allograft is associated with an increase in local tissue expression of both IL-1β and HLA-DR,[18] we looked for the presence of the potent immunosuppressive peptide, TGF-β, in the tissues of the maternal-fetal interface. The TGF-β_1 probe hybridized with a 2.5 kb mRNA species found in both maternal and fetal tissues. TGF-β_1 mRNA expression was increased fivefold ($p < 0.003$) in gestational decidualized endometrium compared with proliferative endometrium and 2.5-fold ($p < 0.002$) compared with secretory endometrium (Fig. 2). Term placental membranes, term placenta, and first-trimester placenta expressed higher levels of TGF-β_1 mRNA when compared with gestational decidualized endometrium (Fig. 3). TGF-β–like bioactivity was detected by normal rat kidney colony formation in soft agar in gestational decidualized endometrium, term placental membranes, and first-trimester and term placenta. Size exclusion chromatography of acid-ethanol extracted proteins resulted in a single broad peak of TGF-β–like bioactivity at approximately 15,000 to 27,000 kd (Fig. 5). Previous studies have shown that under the conditions used TGF-β elutes at a somewhat smaller molecular weight than its true molecular weight of 25,000 kd.[16] Term placenta and term placental membranes had approximately 10 to 20 times more extractable TFG-β bioactivity than did first-trimester decidualized endometrium and term placenta (Fig. 4). Extractable TGF-β bioactivity was not determined in endometrium from the normal menstrual cycle because of the limited amount of tissue available for this study.

Comment

These findings indicate that IL-1β is produced by gestational decidualized endometrium and placental membranes that contain maternal decidua parietalis during the normal course of pregnancy. Both immunoreactive IL-1β and IL-β mRNA levels in endometrium rose significantly during early pregnancy compared with the normal menstrual cycle, suggesting transcriptional regulation of the production if IL-1β in this tissue. Endometrial IL-1β mRNA expression during the normal menstrual cycle first appeared in secretory endometrium beginning on histologic cycle day 23, except for a single proliferative endometrium sample that had evidence of endometritis. Because the process of endometrial decidualization first begins on day 23 of the menstrual cycle,[14] IL-1β may be produced by decidual cells of the endometrial stroma or by the increased numbers of macrophages seen in decidualized endometrium.[4] Unlike maternal decidua, fetal placenta does not produce IL-1β. These findings were unexpected since previous studies have shown that placental tissue explants and isolated cytotrophoblast produce bioactive IL-1 in vitro.[19, 20] It is possible that placenta has the potential to produce IL-1 in vitro given appropriate stimulation, such as adherence to plastic or endotoxin contamination of culture media.[21] Another possibility is that placenta may produce IL-1α and not IL-1β. IL-1α would not be measurable in our IL-1β radioimmunoassay or by our Northern blot analysis. However, IL-1α has the same biologic activities as IL-1β and consequently would be detectable in an IL-1 bioassay. Radioimmunoassay and Northern blot analysis of fresh placenta for IL-1α will be necessary to determine if placenta normally produces this peptide in vivo.

The expression of HLA-DRα, similar to IL-1β, also increased in decidualized endometrium during early pregnancy when compared with endometrium from the menstrual cycle. HLA-DR is composed of an α-subunit and a β-subunit. Because the HLA-DRα subunit has limited polymorphism among most individuals, unlike the highly polymorphic HLA-DRβ subunit, HLA-DRα can be used as a measure of relative HLA-DR expression. These findings are consistent with immunohistochemical localization studies demonstrating an increase in the proportion of HLA-DR–labeled cells in decidua from early pregnancy compared with normal endometrium from the menstrual cycle.[4] It is unclear from this study and previous investigations whether increased HLA-DR expression in decidualized endometrium is a result of increased expression of HLA-DR by resident endometrial cells or is caused by an influx and accumulation of HLA-DR–bearing cells such as macrophages. In addition, there was little, if any, HLA-DR expression in first-trimester placenta, whereas term placenta had marked HLA-DR mRNA expression. Previous immunohistochemical studies would suggest that HLA-DR expression in term placenta is a result of Hofbauer cells of the villous mesenchyme and not the villus trophoblast.[22] Because these cells are separated from maternal decidua by the trophoblast that does not express HLA-DR, allogeneic placental HLA-DR expression probably does not stimulate the local maternal immune response.

Macrophage activation is a predominant feature at sites of immune and inflammatory responses.[5] Macrophages and monocytes express major histocompatibility class II antigens such as HLA-DR, which are important for antigen presentation to T cells. In addition, activated macrophages and monocytes produce a number of cytokines including IL-l, which is an important cofactor in addition to HLA-DR for T-cell activation and recognition of foreign antigens. Antigen-presenting

cells, many of which are tissue macrophages, have been isolated from decidua.[23] The process of endometrial decidualization during early pregnancy is also associated with an increase in the number of macrophages found in this tissue.[4] One possible explanation for our findings is that pregnancy is associated with an influx of activated macrophages into the decidua or that there is an activation of resident decidual macrophages that leads to increased decidual HLA-DR and IL-1β expression.

The beneficial effects that result when the fetal semiallograft provokes an immune reaction by the maternal immune system has been termed "Immunotropism."[24] This theory states that maternal T-cell activation results in the release of various lymphokines that are growth factors for the placenta. Interleukin-3 and granulocyte-macrophage colony-stimulating factor are both secretory products of activated T-cells and stimulate the growth of placental cells in vitro. Because increased decidual IL-1β and HLA-DR expression during pregnancy should lead to local T-cell activation and lymphokine production, our findings are consistent with the immunotropism hypothesis.

Evidence presented in this study that demonstrates TGF-β_1 mRNA and tissue-extractable TGF-β–like bioactivity in decidua, placenta, and placental membranes suggests that this peptide is produced in these tissues. Recent studies have described the potent immunosuppressive properties of TGF-β. The role of TGF-β in regulation of the immune and inflammatory response includes the suppression of lymphokine-activated killer cell activity,[9] inhibition of IL-1–dependent T- and B-cell proliferation,[8] suppression of cytotoxic T-cell activity,[9] and decreased cytotoxicity associated with macrophage activation.[11] On a molar basis, TGF-β is a more potent immunosuppressive agent than cyclosporine.

The beneficial effects of maternal immune recognition without the development of maternal immune rejection of the fetal semiallograft have presented an enigma in the fields of reproduction and transplant immunology. This study has demonstrated that increased IL-1β and HLA-DR expression in maternal endometrium occurs during pregnancy. Although increased IL-1β and HLA-DR expression may be beneficial for T-cell activation and the release of placental immunotrophic growth factors, these are also the initiating events in graft rejection.[18] The production of immunosuppressive peptide TGF-β in the tissues found at the maternal-fetal interface may play a role in the regulation of the maternal immune response and prevent immune rejection of the fetus. The potential role that abnormal production of these immunoregulatory peptides may play in habitual abortion and intrauterine growth retardation is currently being investigated.

REFERENCES

1. Billingston W, Davies M. Maternal antibody to placental syncytiotrophoblast during pregnancy. In: Gill T, Wegmann T, Nisbet-Brown E, Eds. Immunoregulation and fetal survival. New York: Oxford University Press, 1987:15-26.
2. Thomas ML, Harger JH, Wagener DK, Rabin BS, Gill TJ III. HLA antigen sharing and spontaneous abortion in humans. Am J Obstet Gynecol 1985;151:1053-8.
3. Beer AE, Quebbeman JF, Ayers JWT, Haines RF. Major histocompatibility complex antigens, maternal and paternal immune responses and chronic habitual abortions in humans. Am J Obstet Gynecol 1981;141:987-97.
4. Bulmer JN, Johnson PM. Immunohistological characterization of the decidual leucocytic infiltrate related to endometrial gland epithelium in early human pregnancy. Immunology 1985;55:35-44.
5. Unanue E, Allen P. The basis for the immunoregulatory role of macrophages and other accessory cells. Science 1987;236:551-7.
6. Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK. There is more than one interleukin-1. Immunol Today 1986;7:45-56.
7. Romero R, Wu YK, Brody DT, Oyarzun E, Duff GW, Durum SK. Human decidua: a source of interleukin-1. Obstet Gynecol 1989;73:31-4.
8. Wahl SM, Hunt DA, Wong HL, et al. Transforming growth factor-β is a potent immunosuppressive agent that inhibits IL-1 dependent lymphocyte proliferation. J Immunol 1988;140:3026-32.
9. Mule JJ, Schwartz SL, Roberts AB, Sporn MB, Rosenberg SA. Transforming growth factor-β inhibits the in vitro generation of lymphokine-activated killer cells and cytotoxic T-cells. Cancer Immunol Immunother 1988;26:95-100.
10. Rook AH, Rehrl JH, Wakefield LM, et al. Effects of transforming growth factor-β on the functions of natural killer cells; depressed cytolytic activity and blunting of interferon responsiveness. J Immunol 1986;136:3916-20.
11. Tsunawaki S, Sporn M, Ding A, Nathan C. Deactivation of macrophages by transforming growth factor-β. Nature 1988;334:260-2.
12. Frolik CA, Dart LL, Meyers CA, Smith DM, Sporn MB. Purification and initial characterization of a type β transforming growth factor from human placenta. Proc Natl Acad Sci USA 1983;80:3676-80.
13. Derynck R, Jarrett JA, Chen EY, et al. Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. Nature 1985;316:701-5.
14. Noyes RN, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3-25.
15. Chomczynski P, Sacch N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Biochemistry 1987;162:156-9.
16. Assoian RK. Purification of type-β transforming growth factor from human platelets. In: Barnes D, Sirbasku DA, eds. Methods in enzymology. Peptide growth factors. Orlando: Academic Press, 1987:153-62.
17. Roberts AB, Anzano MA, Wakefield LM, Roche NS, Stern DF, Sporn MB. Type β transforming growth factor: a bifunctional regulator of cellular growth. Proc Natl Acad Sci USA 1985;82:119-23.
18. Halloran PF, Cockfield SM, Madrenas J. The mediators of inflammation (interleukin-1, interferon-α, and tumor

necrosis factor) and their relevance to rejection. Transplant Proc 1989;21:26-30.
19. Flynn A, Finke JH, Loftus MA. Comparison of interleukin-1 production by adherent cells and tissue pieces from human placenta. Immunopharmacology 1985;9:19-25.
20. Main EK, Strizki J, Schochest P. Placental production of immunoregulatory factors: trophoblast is a source of interleukin-1. Trophoblast Res 1987;2:149-60.
21. Haskill S, Johnson C, Eierman D, Becker S, Warren K. Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. J Immunol 1988; 140:1090-6.
22. Bulmer JN, Johnson PM. Macrophage populations in the human placenta and amniochorion. Clin Exp Immunol 1984;57:393-403.
23. Elcock JM, Searle RF. Antigen-presenting capacity of mouse decidual tissue and placenta. Am J Reprod Immunol 1985;7:99.
24. Athanassakis I, Bleackley RC, Paetkau U, Guilbert L, Barr PJ, Wegmann TG. The immunostimulatory effect of T cells and T cell lymphokines on murine fetally derived placental cells. J Immunol 1987;138:37-44.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Prevalence and determinants of estrogen replacement therapy in elderly women

Jane A. Cauley, DrPH,[a] Steven R. Cummings, MD,[b, c] Dennis M. Black, PhD,[c] Stephen R. Mascioli, MD,[d] and Dana G. Seeley, MS[c]

Pittsburgh, Pennyslvania, San Francisco, California, and Minneapolis, Minnesota

To better understand which women use estrogen replacement therapy, we examined the prevalence and determinants of estrogen replacement therapy in 9704 nonblack women, age ≥65 years, who participated in the multicenter prospective Study of Osteoporotic Fractures. Overall, 13.7% of women reported current use of oral estrogen; 10.9% took estrogen alone and 2.8% took estrogen opposed by progestin. Four percent currently used parenteral estrogen compounds. Current use declined sharply with age from 17% at age 65 to 4% at age ≥85. The primary determinant of estrogen replacement therapy was the type of menopause; the odds of using estrogen replacement therapy in current users compared with never users were approximately five times higher in women with a surgical menopause. Estrogen use was more common among women who had higher levels of education and were less obese. Furthermore, estrogen replacement therapy users were more likely to drink alcohol and to participate in sports and recreation. A diagnosis of osteoporosis was the major determinant of continued estrogen use, but only 24% of women with a diagnosis of osteoporosis used estrogen replacement therapy. We conclude that only a small proportion of elderly women in the United States use estrogen replacement therapy. Selection factors for use of estrogen are evident and may introduce bias in studies of estrogen and disease. In consideration of the distribution of these selection factors, estrogen users will tend to be at lower risk of coronary disease and possibly breast cancer but at greater risk for hip fractures. (Am J Obstet Gynecol 1990;163:1438-44.)

Key words: Estrogen replacement therapy, osteoporosis, coronary disease, breast cancer

Osteoporosis contributes to an estimated 1.5 million fractures per year[1] at an annual cost of at least 7 to 10 billion dollars in the United States.[2] Because the number of individuals 65 years or older will continue to rise, the number of fractures is likely to double by the end of the century.[1] The International Consensus Conference on Osteoporosis[3] concluded that estrogen replacement therapy was the only well-established preventive measure that could reduce the number of osteoporotic fractures.

Coronary heart disease is the leading cause of death in older women and accounts for a large proportion of illness and disability in women 65 years of age and older.[4] Most studies suggest that postmenopausal estrogen use substantially reduces morbidity and mortality from coronary heart disease,[5] although not all studies have observed such benefit.[6]

Because of the potential for prevention of two major causes of morbidity and mortality in elderly women, better understanding of the prevalence of long-term estrogen use in elderly women, as well as the determinants of long-term estrogen use, is essential to assess its public health impact.

Prescriptions for estrogen increased sharply from 1966 to 1975,[7, 8] with the number of dispensed estrogen prescriptions peaking at 28 million per year in 1975, followed by a decline in use until 1981. After the relationship between estrogen replacement therapy and endometrial cancer was reported in 1975,[9] prescriptions for estrogen declined to a low of 14 million in 1980. Since 1981, use of estrogen replacement therapy in the United States has been steadily increasing.[7, 8] Twenty-one million estrogen prescriptions were dispensed in 1986.[8] Most of the previous research on the prevalence of estrogen replacement therapy has examined prevalence rates before 1975,[10-12] and have primarily included women <age 60. Usage rates in elderly women tend to be much lower and to decline with age,[12, 13] but there are few data regarding use of estrogen by older women. The highest rates of use were

From the Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh,[a] the Division of General Internal Medicine[b] and the Department of Epidemiology and Biostatistics,[c] University of California at San Francisco, and the Division of Epidemiology, School of Public Health, University of Minnesota.[d]

Supported by grants AG05047 AR35583, AG05394, AR35582, and AM35584 from the National Institutes of Health. Dr. Cummings' work is supported, in part, by the Henry J. Kaiser Family Foundation Faculty Fellowship in General Internal Medicine.

This article is a publication of the Study of Osteoporotic Fractures Research Group. Members and institutional affiliations are listed at the end of the article.

Received for publication March 13, 1990; revised July 5, 1990; accepted July 16, 1990.

Reprint requests: Jane A. Cauley, DrPH, A524 GSPH, Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA 15261.

6/1/23821

observed in upper socioeconomic populations[11, 13] and in whites, compared with both blacks[14] and Mexican-Americans.[15] Geographically, rates tended to be higher in the west than in the east.[8]

Estrogen replacement therapy is not randomly distributed in the population; this could account for some of the observed effects of estrogen replacement therapy on disease outcomes. Estrogen replacement therapy users tended to be less obese,[11, 13] more highly educated,[12] and were more likely to have undergone a surgical menopause.[13] There has been no research on the determinants of estrogen replacement therapy in relationship to potential risk factors for osteoporosis.

In this study, we examined the prevalence and determinants of estrogen replacement therapy in a large cohort of elderly women who participated in the multicenter Study of Osteoporotic Fractures from 1986 to 1988.

Methods

The Study of Osteoporotic Fractures is a prospective multicenter study of risk factors for hip and Colles' fractures in a cohort of 9704 nonblack women aged 65 and older. Subjects were recruited from Oct. 1, 1986, to Oct. 31, 1988, from population-based lists in four areas across the United States: Baltimore, Md., Minneapolis, Minn., Portland, Ore., and the Monongahela Valley near Pittsburgh, Pa. We excluded black women, women with bilateral hip replacement, and those planning to relocate.

Detailed information was collected at baseline on use of estrogen and progestin. "Current" users were women who reported use at the time of the interview. Women were asked to bring all medications to the clinic for verification of use, preparation, and dosage. Information was collected regarding oral and parenteral (skin patches, injections, vaginal creams, suppositories) estrogens and oral progestins. To estimate duration of use, women were asked to check all ages of a chart that listed ages 40 through 100 in which estrogen replacement therapy was used. Duration of use was then calculated by adding the total number of ages that a woman used estrogen replacement therapy.

In addition to estrogen use, the following variables were assessed by questionnaire and interview: medical and reproductive history, personal and family fracture history, alcohol consumption, cigarette smoking, physical activity, demographics, and height and weight at age 25 and at age 50. Women were considered to have had a surgical menopause if they reported a bilateral oophorectomy at the age they stopped menstruating. A positive response to whether a physician had ever told them that they had osteoporosis or brittle bones was interpreted as a previous diagnosis of "osteoporosis." Current body weight was measured on a standard balance beam scale without shoes and heavy outer clothing. Height was measured without shoes with a Harpenden Stadiometer. Waist girth was measured at the midpoint between the bottom of the rib cage and the top of the iliac crest. Hip girth was measured at the widest point—usually at the level of the greater femoral trochanter. Women were considered obese if their body mass index was ≥ 27.3.[16] Women were considered to be upper body obese if their waist to hip ratio was ≥ 0.84.[17]

Statistical analyses were performed using the SAS statistical package.[18] The statistical significance of the univariate association of the dichotomous risk factors to estrogen use was assessed by χ^2 test of the equality of the proportions of women with the risk factor in the three categories of estrogen use.

To adjust for confounding, multivariate logistic analyses were performed. We included as predictor variables selected predictors with a univariate $p < 0.001$, or those that have been shown to have a strong association with estrogen usage in previous research. The same set of predictors was used in four multivariable logistic analyses: current versus never, current versus past for surgical and natural menopause.

Results

A total of 9704 women enrolled in the Study of Osteoporotic Fractures. Characteristics of the participants are described in Table I. The mean age of the subjects was 71.7 years. The majority of the women reported a natural menopause. Information on type of menopause was missing in approximately 5% of the women, and hence, these women are excluded from the multivariate analyses.

Overall, 13.7% of the women reported current use of oral estrogens, and 4% reported current use of parenteral estrogens (Table II). The largest percentage of parenteral users used vaginal estrogen compounds. Of the women currently taking oral preparations (13.7%), 10.9% were taking unopposed estrogen replacement therapy, and 2.8% were treated with combined estrogen-progestin therapy.

The percent of current users of unopposed estrogen therapy varied by type of menopause and by hysterectomy status. Only 9.4% of current users who reported a surgical menopause were taking estrogen replacement therapy opposed by progestins, compared with 25% in women with a natural menopause.

About 4% of women currently using oral estrogen replacement therapy were also using vaginal estrogen. Twenty-seven percent reported past oral use and 12% reported past parenteral use. Current users tended to have initiated oral use at a later age than past users. Current users had taken oral estrogen replacement therapy for an average of 14.8 ± 10.5 years. Current

Table I. Baseline characteristics of subjects

Characteristic	*n*	%
No.	9704	
Age, mean (yr)	71.7 ± 5.3	
65-69	4112	42
70-74	3041	31
75-79	1546	16
80-84	773	8
≥85	232	2
Type of menopause		
Surgical	1092	11
Natural	8153	84
Unknown	459	5
Hysterectomy		
Yes	3871	40
No	5814	60

Table II. Prevalence of use of oral and parenteral estrogen and descriptive statistics on age at first use and duration of use

Oral estrogen therapy		
Current users (*n*, %)		1330 (13.7%)
Estrogen only	1055 (10.9%)	
Estrogen plus progestin	275 (2.8%)	
Past users (*n*, %)		2603 (27.0%)
Never users (*n*, %)		5771 (59.5%)
Age at first use (yr, mean ± SD)		
Current users		52.1 ± 10.7
Past users		48.8 ± 7.7
Duration of use (yr, mean ± SD)		
Current users		14.8 ± 10.5
Past users		5.2 ± 6.4
Parenteral estrogen therapy*		
Current users (*n*, %)		388 (4.0%)
Past users (*n*, %)		1156 (11.9%)
Never users (*n*, %)		8160 (84.1%)
Age at first use (yr, mean ± SD)		
Current users		62.7 ± 9.2
Past users		53.0 ± 11.8
Duration of use (yr, mean ± SD)		
Current users		6.0 ± 7.2
Past users		2.6 ± 4.8

*Parenteral: Estrogen use by cream, patch, suppository, or injection.

parenteral therapy was started at a later age than oral estrogen therapy.

Use of oral estrogens declined with age (Fig. 1). There was about 40% difference in use among women age 65 to 69 years compared with women age 75 to 79 years. Only 4% of women age 85 and older were currently using oral estrogens. The χ^2 test on the differences in the proportion of current and past users by age was statistically significant, $\chi^2 = 245.6$, $p < 0.0001$.

Current users were more likely to be currently married, to have more years of education, if married, to have a husband with more years of education, to have had a hysterectomy, to have undergone a surgical menopause, to report drinking alcohol, to have ever smoked, to participate in physical activity, to take walks for exercise, and to have ever been pregnant (Table III). Users were less likely to be obese and, more specifically, less likely to be upper body obese. Even at ages 25 and 50 years, current users weighed less than either past users or never users (data not shown).

Although women diagnosed as osteoporotic were more likely to be using estrogen, there was little difference in the proportion of users by whether or not the participant reported any previous fracture after age 50. Users were less likely to report a previous wrist fracture. Women with either a mother or father with a history of fracture after age 50 were more likely to report current or past use of estrogen replacement therapy.

Multiple logistic analyses revealed that the primary determinant of current estrogen replacement therapy use was type of menopause; women with a surgical menopause were more likely to report current estrogen replacement therapy (odds ratio, 4.8; 95% confidence interval, 4.11 to 5.81) compared with never users and were also more likely (odds ratio, 2.30; 95% confidence interval, 1.94 to 2.74) to continue estrogen replacement therapy (current users compared with past users). Because of this strong effect and because of possible bias from selection factors for surgical menopause, we stratified women by type of menopause in subsequent analyses.

In women who had undergone a surgical menopause, current users, compared with never users (Table IV), were younger and were 40% more likely not to be obese, to have greater than a twelfth grade education, and to drink alcohol. In this group, after age, the only significant predictor of continued estrogen replacement therapy (comparing current with past use) was having been diagnosed as osteoporotic.

In women with a natural menopause, a comparison of current with never users with respect to age, education, obesity, and alcohol consumption showed similar results. In addition to these factors, current users were also more likely to have participated in physical activity. However, the strongest determinant of current estrogen replacement therapy in the natural menopause group was having been given a diagnosis of osteoporosis by a physician; current users were 2.8 times as likely to have had a diagnosis of osteoporosis. This diagnosis was also important for determining continued long-term use of estrogen replacement therapy.

Comment

We found that the overall prevalence of estrogen replacement therapy among elderly women is low and decreases with age.

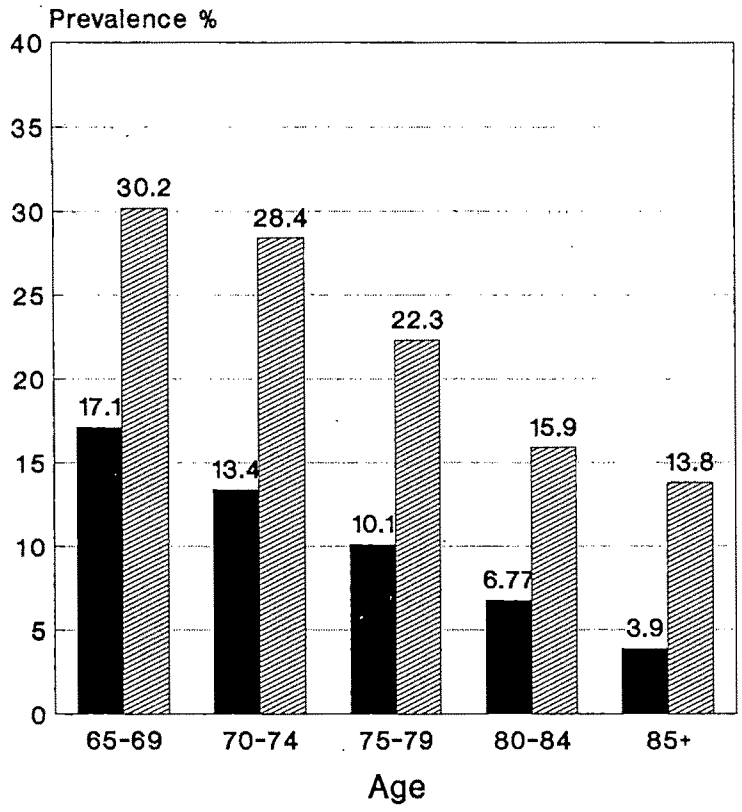


Fig. 1. Prevalence of use of oral estrogen replacement therapy by age, Study of Osteoporotic Fractures (n = 9704). *Solid bars*, Current use; *hatched bars*, Past use.

We recruited subjects from population-based listings in four cities across the United States. However, participants in the Study of Osteoporotic Fractures are not a representative sample of older women; they are volunteers who are likely to be healthier and perhaps more health conscious than those who did not participate. Consequently, our prevalence rates may overestimate rates of use among the general population.

Earlier studies have found determinants of estrogen replacement therapy in younger postmenopausal women. In this study we confirmed that selection factors for estrogen replacement therapy also exist in elderly women. These estrogen replacement therapy characteristics would presumably lower the risk of coronary heart disease, and possibly breast cancer, but increase risk of hip fracture; hence, failure to consider these characteristics leads to bias in studies of the effects of estrogen replacement therapy on coronary heart disease, hip fracture, and breast cancer.

Our results are consistent with previous reports that education level,[14, 15] degree of obesity,[11, 13] alcohol consumption, and surgical menopause[13] are important determinants of estrogen use. Surgical menopause was the strongest predictor of estrogen replacement therapy use in this population of elderly women. We confirmed the results of other studies with regard to no difference in smoking status by estrogen replacement therapy use.[11, 13] Users of estrogen replacement therapy were somewhat more likely to participate in sports and recreation, although this observation was confined to women with natural menopause. This may reflect their higher socioeconomic status and their lower degree of obesity.

Most previous studies on the determinants of estrogen replacement therapy focused primarily on cardiovascular disease risk factors; this study also included possible risk factors for osteoporosis. A diagnosis of osteoporosis by a physician was a significant determinant of current estrogen replacement therapy in women with a natural menopause. Of importance, it was also the strongest determinant of continued long-term estrogen replacement therapy in both the natural and surgical menopause groups; however, the prevalence of estrogen replacement therapy (24%) was low in this potentially high-risk group.

The lack of a relationship between estrogen replacement therapy and previous hip fractures may be related to failure of orthopedists to prescribe estrogen replacement therapy, perhaps because these women are not considered osteoporotic or because many women cannot tolerate or refuse estrogen replacement therapy. Estrogen replacement therapy users were less likely

Table III. Risk factor distribution by estrogen use status

	Current	*Past*	*Never*	*p**
Demographics				
% Currently married	57.7	53.3	44.7	<0.001
% With < high school education	15.8	18.2	27.0	<0.001
% With husband with < high school education	25.5	30.2	40.1	<0.001
Fracture history (% yes)				
Fracture after age 50	34.8	35.6	38.3	0.01
Hip fracture after age 50	2.0	1.6	2.0	0.46
Wrist fracture after age 50	7.6	10.0	12.9	<0.001
Mother fractured a bone after age 50	26.6	25.8	21.2	<0.001
Father fractured a bone after age 50	11.3	12.1	10.5	0.24
Medical history				
% With osteoporosis	24.6	14.3	12.8	<0.001
% With fracture of spine	9.7	5.4	5.9	<0.001
Reproductive history				
% Ever pregnant	87.4	84.6	82.6	<0.001
% Ever had hysterectomy	72.2	44.3	30.6	<0.001
% Surgical menopause	27.0	14.2	7.3	<0.001
Smoking				
% Ever smoked	43.2	43.5	37.0	<0.001
Physical activity				
% Participate in physical activities in past week	75.5	73.5	63.0	<0.001
% Work up a sweat at least once a week	42.0	39.6	32.0	<0.001
% Take walks for exercise	56.8	54.4	46.7	<0.001
Alcohol use				
% Drink alcohol	76.1	74.8	66.4	<0.001
Body mass index				
% Body mass index ≥ 27.3 (obese)	28.6	36.2	41.1	<0.001
Waist/hip ratio				
% Waist/hip ratio >0.84 (upper body obese)	28.1	29.8	33.5	<0.001

*χ^2 test based on 2 × 3 tables.

to report a previous wrist fracture, probably reflecting a protective effect of prior estrogen replacement therapy.

Parental fracture history had a stronger influence on hormone use than personal fracture history. This may be a result of study bias: better educated estrogen users may be more likely to know and report their family history of fracture. It may also relate to the role of women as caregivers since these women may have taken care of elderly parents after fractures and personally experienced the fracture-related decline in functional status or death of a parent.

In an observational population-based study of the effect of estrogen replacement therapy on a disease outcome, the observed low prevalence of estrogen replacement therapy would imply that sample sizes must be large to have adequate statistical power to see a significant effect. Selection factors differ and risk of disease differs in women with a natural versus surgical menopause. Hence, stratification by type of menopause is necessary in evaluating estrogen replacement therapy, and will tend to increase sample size requirements.

Previous research has suggested that some of the protective effects of estrogen replacement therapy on hip fracture and coronary heart disease may be lost within a few years of discontinuing estrogen replacement therapy.[19, 20] For example, Weiss et al.[19] found a significant reduction in risk of hip and wrist fractures in current users only. Discontinuation of estrogen replacement therapy 1 to 2 years before the fracture offered no significant effect. For coronary heart disease,[20] the odds ratio was lower for current users than ever users. Hence, effective prevention of hip fracture and coronary heart disease with estrogen may require

Table IV. Independent predictors of estrogen use stratified by type of menopause: Multiple logistic analysis

Variable	Unit of measurement	*Current use vs never use*				*Current use vs past use*			
		Surgical menopause (n = 731)		*Natural menopause (n = 5938)*		*Surgical menopause (n = 677)*		*Natural menopause (n = 2997)*	
		OR	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Age	5 yr	**0.62**	**0.60-0.64**	**0.68**	**0.67-0.69**	**0.84**	**0.81-0.88**	**0.86**	**0.84-0.88**
Education	0 = ≥12th grade 1 = <12th grade	**0.63**	**0.44-0.91**	**0.60**	**0.49-0.74**	0.79	0.54-1.15	0.86	0.68-1.09
Obesity	0 = Not obese 1 = Obese	**0.65**	**0.47-0.89**	**0.58**	**0.49-0.69**	0.84	0.61-1.16	**0.68**	**0.57-0.81**
Alcohol in past 30 days	0 = No 1 = Yes	**1.54**	**1.13-2.10**	**1.38**	**1.05-1.61**	1.06	0.77-1.45	1.08	0.91-1.28
Diagnosis of "osteoporosis"	0 = No 1 = Yes	1.37	0.93-2.03	**2.85**	**2.39-3.42**	**1.62**	**1.08-2.42**	**2.32**	**1.90-2.82**
Physical activity in past week	0 = No 1 = Yes	1.33	0.95-1.85	**1.59**	**1.34-1.89**	0.86	0.61-1.22	1.16	0.96-1.40
Maternal history of fracture after age 50	0 = No 1 = Yes	1.01	0.71-1.45	1.13	0.96-1.34	0.96	0.68-1.37	0.98	0.81-1.17

Boldface type indicates statistical significance. *OR,* Odds ratio; *CI,* confidence interval.

long-term use of estrogen replacement therapy into the seventh, eighth, or even ninth decade of life.

Our data show that few elderly women currently use estrogen replacement therapy. Future research should identify the barriers to long-term use of estrogen replacement therapy. Some possible barriers to estrogen replacement therapy include fear of cancer, unwanted side effects, cost, and polypharmacy (i.e., elderly women may take many other medications for immediate management of medical conditions, and may not want to take one for prophylaxis). It may also reflect physician practices and beliefs. Because of the uncertainty over whether long-term estrogen replacement therapy is associated with breast cancer, physicians may be unwilling to recommend long-term estrogen replacement therapy. If a treatment is known to be effective but is unacceptable to most elderly women, then alternative strategies for prevention must be developed.

Investigators in the Study of Osteoporotic Fractures Research Group are as follows:

University of California, San Francisco (coordinating center): S. R. Cummings (principal investigator), M. C. Nevitt (project director), D. Black (study statistician), H. K. Genant (director, central radiology laboratory), C. Arnaud, C. Brown, W. Browner, C. Fox, S. Harvey, S. B. Hulley, D. Seeley, P. Steiger

University of Maryland: R. Sherwin (principal investigator), J. Scott (project director), B. Hohman (clinic coordinator), J. Lewis, E. Oliner, L. Schools

University of Minnesota: R. Grimm, Jr. (principal investigator), S. Mascioli (project director), C. Bell (clinic coordinator), R. Prineas, K. Jacobson, S. Enebo, E. Mitson, L. Stocke, M. Oliver, D. Thomas

University of Pittsburgh: L. H. Kuller (principal investigator), J. A. Cauley (project director), M. Nasim (clinic coordinator), C. Bashada, L. Buck, A. Githens, L. Harper (follow-up data coordinator), D. Medve

The Kaiser Permanente Center for Health Research, Portland, Oregon: T. M. Vogt (principal investigator), W. M. Vollmer, J. Blank (project director), H. Glauber, T. Marino, R. Bright, B. Packer

REFERENCES

1. Riggs BL. Pathogenesis of osteoporosis. Am J Obstet Gynecol 1987;156:1342-6.
2. Kelsey JL, Hoffman S. Risk factors for hip fracture. New Engl J Med 1987;316:404-6.
3. Osteoporosis [Editorial]. Lancet 1987;2:833-5.
4. Feinleib M, Gillum RG. Coronary heart disease in the elderly: the magnitude of the problem in the United States. In: Wenger NK, Furberg CD, Pitt E, eds. Coronary heart disease in the elderly. Amsterdam: Elsevier, 1986:25-9.
5. Bush TL, Barrett-Connor E. Noncontraceptive estrogen use and cardiovascular disease. Epidemiol Rev 1985;7:80-103.
6. Wilson PWF, Garrison RJ, Castelli WP. Postmenopausal estrogen use, cigarette smoking, and cardiovascular morbidity in women over 50. The Framingham study. N Engl J Med 1985;313:1038-43.
7. Kennedy DL, Baum C, Forbes MB. Noncontraceptive estrogens and progestins: use patterns over time. Obstet Gynecol 1985;65:441-6.
8. Hemminki E, Kennedy DL, Baum C, McKinlay SM. Pre-

scribing of noncontraceptive estrogens and progestins in the United States, 1974-86. Am J Public Health 1988; 78:1479-81.
9. Mack TM, Pike MC, Henderson BE, et al. Estrogens and endometrial cancer in a retirement community. N Engl J Med 1975;294:1262-7.
10. Markush RE, Turner SL. Epidemiology of exogenous estrogens. HSMHA Health Rep 1971;86(1):74-86.
11. Barrett-Connor E, Brown WV, Turner J, Austin M, Criqui MH. Heart disease risk factors and hormone use in postmenopausal women. JAMA 1979;241:2167-9.
12. Pfeffer RI. Estrogen use in postmenopausal women. Am J Epidemiol 1977;105:21-9.
13. Barrett-Connor E, Wingard DL, Criqui MH. Postmenopausal estrogen use and heart disease risk factors in the 1980s. JAMA 1989;261:2095-100.
14. The Lipid Research Clinics Population Studies Data Book. Vol. 1. The Prevalence Study. US Department of Health and Human Services. Public Health Service, National Institutes of Health, July 1980; NIH publication no 80-1527.
15. Stern MP, Brown BW, Haskell WL, Farquhar JW, Wehrle CL, Wood PDS. Cardiovascular risk and use of estrogens or estrogen-progestogen combinations. Stanford three-community study. JAMA 1976;235:811-5.
16. National Center for Health Statistics. Najjar MF, Roland M. Anthropometric reference data and prevalence of overweight, United States, 1976-1980. DHHS publication no (PHS) 87-1688. Public Health Service, Washington, DC: US Government Printing Office, Oct 1987; (Vital and health statistics; data from the National Health Survey; series 11; no 238).
17. Maugh TH. A new marker for diabetes. Science 1982; 215:651.
18. SAS Institute Inc. SAS user's guide: statistics, version 5. Cary, North Carolina; SAS Institute, Inc, 1985.
19. Weiss NS, Ure CL, Ballard JH, et al. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med 1980;303:1195-8.
20. Pfeffer RI, Whipple GH, Kurosaki TT, et al. Coronary risk and estrogen use in postmenopausal women. Am J Epidemiol 1978;107:479-87.

Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta

Paul Y. C. Cheung. BSc,[a] John C. Walton, MD,[b] Hsin-Hsiung Tai, PhD,[c] Simon C. Riley, PhD,[a] and John R. G. Challis, PhD, DSc[a]

London, Ontario, Canada, and Lexington, Kentucky

Biochemical studies have shown the presence of type I oxidized nicotinamide-adenine dinucleotide–dependent 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta. However, the localization of prostaglandin dehydrogenase within these tissues is not known. Because the distribution of prostaglandin dehydrogenase may affect the concentration of prostaglandins that reach the myometrium, we used immunocytochemistry to localize immunoreactive prostaglandin dehydrogenase in fetal membranes and placenta. We also examined whether this distribution changed with labor. Tissues were collected at term elective cesarean section or after term spontaneous labor and delivery, were fixed, embedded, and sectioned at 5 μm. Immunoreactive prostaglandin dehydrogenase was determined with a polyclonal primary antibody to human placental prostaglandin dehydrogenase and visualized with the avidin-biotin procedure. Epithelial and epithelium-derived cells were identified by positive staining with a polyclonal antikeratin primary antibody. Cytokeratin staining was observed in amniotic epithelium, trophoblast layer of chorion, invading trophoblast in decidua, and all subsets of trophoblast in the placenta. Immunoreactive prostaglandin dehydrogenase was localized to the trophoblast layer of chorion, invading trophoblast in decidua, and in syncytiotrophoblast and intermediate trophoblast but not cytotrophoblast in the placenta. In chorion, approximately 50% to 60% of the nonvacuolated trophoblast cells stained positively for prostaglandin dehydrogenase. There was no change in the localization of immunoreactive prostaglandin dehydrogenase in any tissue in association with labor. (AM J OBSTET GYNECOL 1990;163:1445-9.)

Key words: Prostaglandin, metabolism, immunohistochemistry, fetal membranes, placenta

Prostaglandins are strongly implicated in the sequence of events leading to myometrial contractility and parturition at term.[1, 2] In women, amnion and decidua have been identified as major sites of prostaglandin synthesis, whereas the chorion is a major site of prostaglandin metabolism.[3-5] This pattern is reflected in the higher specific activity of the oxidized nicotinamide-adenine dinucleotide–dependent 15-hydroxyprostaglandin dehydrogenase (PGDH) in chorion and placenta compared with the amnion and decidua.[6, 7] This enzyme converts prostaglandins (prostaglandin E_2 and prostaglandin $F_{2\alpha}$) to the 15-keto metabolites, the initial step in inactivating primary prostaglandins. Although previous studies have suggested that small amounts of primary prostaglandins may cross full-thickness membranes without metabolism,[8, 9] the significance of this observation has been questioned because of the high PGDH activity in chorion. However, chorion is a heterogeneous tissue,[10, 11] and it seemed possible that PGDH was not distributed uniformly throughout the tissue. Hence we used immunocytochemistry to determine the distribution and localization of immunoreactive PGDH in the amnion, chorion, decidua, and placenta and determined whether there were changes in the pattern of staining with labor at term.

From the Department of Obstetrics and Gynecology and the Department of Physiology, University of Western Ontario, the Lawson Research Institute, and St. Joseph's Health Centre,[a] the Department of Pathology, St. Joseph's Health Centre and University of Western Ontario,[b] and the College of Pharmacy, University of Kentucky, Lexington.[c]

Supported by the Medical Research Council of Canada (group grant in Fetal and Neonatal Health and Development, J.R.G.C.).

Received for publication April 6, 1990; revised July 2, 1990; accepted July 17, 1990.

Reprint requests: Paul Cheung, Lawson Research Institute, St. Joseph's Health Centre, University of Western Ontario, 268 Grosvenor St., London, Ontario, Canada N6A 4V2.

6/1/23862

Material and methods

Collection of tissues. Placentas with membranes and adherent decidua were obtained from uncomplicated pregnancies at either term elective cesarean section before labor ($n = 5$) or at term spontaneous delivery ($n = 5$). Tissues were fixed in either 10% neutral buffered formalin or Bouin's solution for 4 hours, then

washed and stored in 70% ethanol. There was no difference in immunostaining with the different fixatives. The membranes were then rolled and the placenta trimmed before embedding in paraffin wax. The paraffin blocks were then sectioned at 5 μm.

Immunocytochemical analysis. The sections were stained for immunoreactive-PGDH according to the avidin-biotin procedure (Vector ABC; Vector Laboratories, Burlingame, Calif.). Briefly, the sections were deparaffinized with three washes of xylene substitute (BDH Chemicals, Toronto) and rehydrated in serial washes of ethanol, and phosphate-buffered saline solution (0.01/mol/L, pH 7.5). Endogenous peroxidase activity was reduced by the exposure of the sections to 3% hydrogen peroxide in water for 30 minutes. The sections was counterstained with Carrazzi's hematoxylin, which turns the nucleus blue. Nonspecific staining was reduced by incubating the sections in 10% goat serum for 20 minutes. The sections were then incubated overnight with the primary antibody or the appropriate substituted control (see below). The polyclonal primary antibody (1:2000 and 1:3000, Cayman Laboratories, Ann Arbor) was raised in rabbits against purified human placental PGDH. Additional sections were also stained for cytokeratin, which is an epithelial cell marker. The polyclonal primary antibody (antikeratin, wide-spectrum screening; 1:1000, Dako Corp., Santa Barbara) was raised in rabbits against bovine muzzle epidermal keratin. All antibodies were diluted in buffer (1 gm bovine serum albumin, 20 mg sodium azide, 100 ml phosphate-buffered saline solution).

After overnight incubation (18 hours), sections were washed with phosphate-buffered saline solution and incubated in the presence of a biotinylated goat anti-rabbit IgG antibody (1:500) for 1 hour. The sections were then washed, incubated with Vectastain (Vector ABC) for 2 hours and immunoreactive PGDH was visualized by exposure to 3,3′-diaminobenzidine tetrahydrochloride [6 mg dissolved in 4 ml Tris buffer (0.05 mol/L, pH 7.5) with 3 drops of 3% hydrogen peroxide]. The sections were dehydrated and mounted (Permount; Fisher Scientific, Toronto). Sections were examined by means of light microscopy.

It has been suggested previously that the trophoblast layer of chorion at term consists of two subpopulations of trophoblast, vacuolated and nonvacuolated.[10] In chorion, the number of vacuolated and nonvacuolated cells in the trophoblast layer staining positive for immunoreactive PGDH was quantified using the Bioquant System IV (Bioquant; Nashville; field = 0.175 mm^2 at ×250 magnification).[11] Five randomly selected fields were counted in stained sections from each patient and the proportion of stained cells was expressed as a percentage of the total vacuolated and nonvacuolated trophoblasts. Student's *t* test was used to seek significant differences in the number of stained vacuolated and nonvacuolated trophoblast between cesarean section and spontaneous labor tissues.

Controls. Three sets of negative controls were used in this study. Tissue sections were incubated either with antibody dilution buffer in the absence of primary antibody, with nonimmune rabbit serum (1:2000 and 1:3000 dilution), or with primary antibody that had been preabsorbed with purified PGDH[12] overnight. Before use, the preabsorbed antibody was centrifuged at 178,000 *g* for 30 minutes at 4° C, and the supernatant fraction was substituted for the primary antibody in the overnight incubation.

Results

Amnion (Fig. 1, *A* and *C*). In the amnion, positive staining for cytokeratin (data not shown) was found in the epithelial layer but not in the subepithelial layers of tissues obtained from cesarean section or spontaneous labor and delivery. No positive staining for immunoreactive PGDH was observed in the epithelial or subepithelial layers of the amnion in patients undergoing either term elective cesarean section or spontaneous labor and delivery.

Chorion-decidua (Fig. 1, *A* and *C*). Positive staining for cytokeratin (data not shown) was observed only in the trophoblast layer of chorion and in trophoblast cells that had invaded decidua. No staining was found in the reticular or cellular layers of chorion.[13] Positive immunoreactivity for PGDH was observed only in the trophoblast layer of chorion. However, only 50% to 60% of nonvacuolated and <20% of vacuolated trophoblast showed positive immunostaining. There was no significant difference in the percentages of vacuolated and nonvacuolated trophoblast that showed positive immunoreactivity for PGDH between cesarean section and spontaneous labor tissues (Table I). No PGDH immunostaining was found in the reticular or cellular layers or in the degenerative villi of the chorion.

No staining was observed for immunoreactive PGDH in the stromal cells in the decidua. However, trophoblast that had invaded decidua did show positive staining for PGDH. Positive staining for immunoreactive PGDH was eliminated in the negative controls [preabsorption of primary antibody with excess purified antigen (Fig. 1, *B*), antibody dilution buffer without primary antibody, and substitution of primary antibody with nonimmune rabbit serum].

Placenta (Fig. 1, *D*). Positive staining for cytokeratin (data not shown) was found in syncytiotrophoblast, cytotrophoblast, and intermediate trophoblast[14, 15] in both villous and extravillous sites. Intense immunopositive PGDH staining was observed in intermediate tropho-

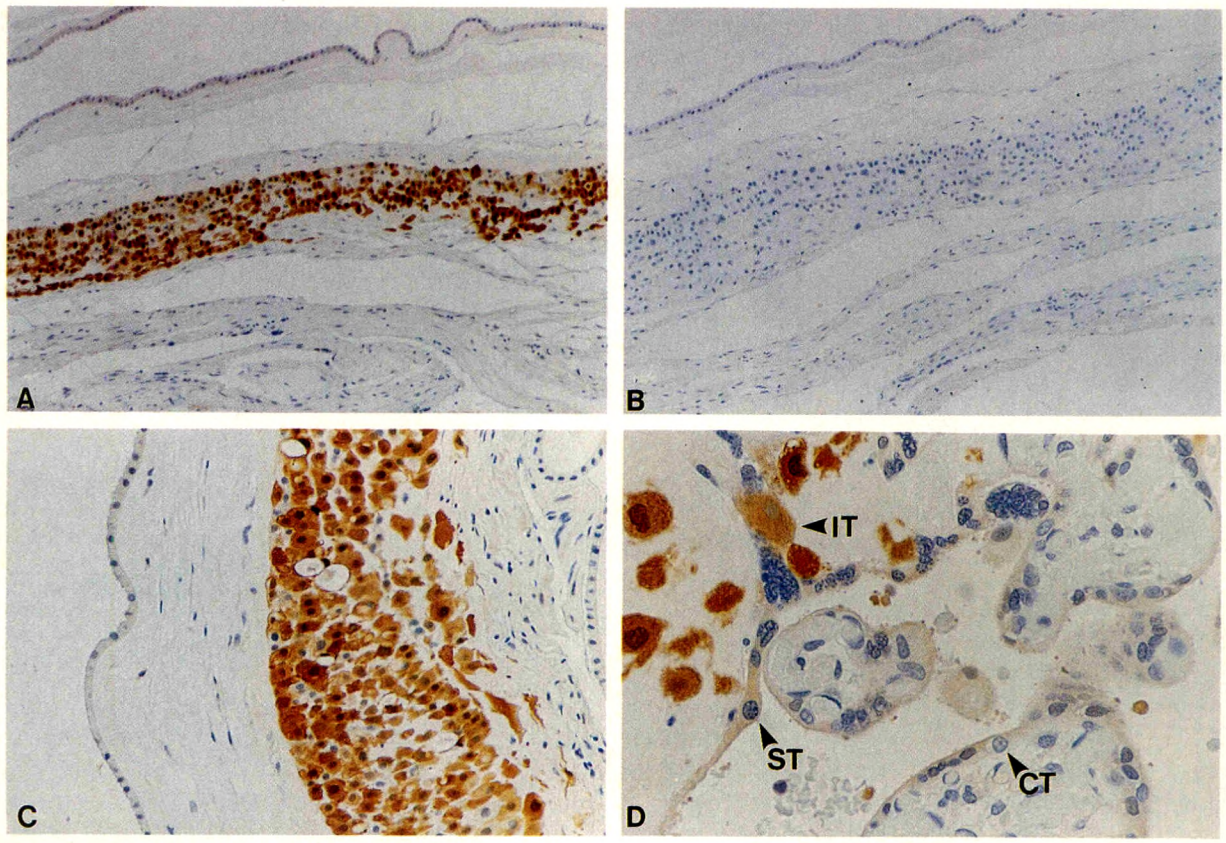


Fig. 1. Staining for PGDH in human fetal membranes, decidua, and placenta. **A,** Full-thickness membranes stained with PGDH primary antibody showed immunoactivity in chorion and invading trophoblast in decidua **(B)** that was abolished by preabsorbing the primary antibody with purified PGDH. **C,** Higher-power section through membranes shows vacuolated and nonvacuolated trophoblast staining. **D,** Section through placenta shows positive immunostaining in intermediate trophoblast *(IT)*, syncytiotrophoblast *(ST)*, and lack of staining in cytotrophoblast *(CT)*. (Original magnifications: **A** and **B,** ×67; **C,** ×133; **D,** ×267.)

blast cells in villous and extravillous sites. Weak positive staining was found in syncytiotrophoblast. Positive immunostaining was abolished in all negative controls. We did not observe PGDH immunostaining in the cytotrophoblast, villous core tissue, or fetal endothelial tissue. Similar patterns of PGDH staining were observed in placentas obtained at both cesarean section and spontaneous labor and delivery.

Comment

This study reports, for the first time, localization of immunoreactive PGDH in the fetal membranes, decidua, and placenta. In the membranes PGDH was present in the trophoblast layer of chorion, in approximately 55% of the nonvacuolated cells, but was absent from amnion and decidual stromal cells. In the placenta PGDH was localized to intermediate trophoblast, weakly to syncytiotrophoblast, but was absent from cytotrophoblast.

Table I. The percentage of vacuolated and nonvacuolated trophoblast cells showing positive immunoactivity for PGDH in the trophoblast layer of the chorion laeve*

Cell type	*Percentage*	
	Spontaneous labor plus delivery (n = 5)	*Cesarean section (n = 5)*
Vacuolated trophoblast	10.0 ± 2.0	15.0 ± 7.0
Nonvacuolated trophoblast	55.0 ± 4.0	53.0 ± 4.0
Total vacuolated trophoblast cells in trophoblast layer	26.0 ± 6.0	10.0 ± 4.0
Total nonvacuolated trophoblast cells in trophoblast layer	74.0 ± 6.0	90.0 ± 4.0

*The percentage of vacuolated and nonvacuolated trophoblast cells occupying the trophoblast layer is also shown (mean ± SEM).

We used cytokeratin staining to identify epithelial and epithelial-derived cells, including trophoblast.[16] Colocalization of PGDH and cytokeratin was not attempted in this study; therefore we cannot state definitively whether all invading trophoblast in the decidua or intermediate trophoblast in the placenta[14, 15] stained for PGDH. However, the cells staining immunopositive for PGDH in these locations had morphologic characteristics of intermediate trophoblast. Intermediate trophoblast also stain positively for human placental lactogen and pregnancy-specific β_1-glycoprotein, but not for human chorionic gonadotropin, thereby distinguishing them from cytotrophoblast and syncytiotrophoblast.[14] We believe that the PGDH staining observed in this study is specific because it was absent after preabsorbing the primary PGDH antibody with a purified PGDH preparation, and no positive staining was found if the primary antibody was omitted, or replaced with nonimmune rabbit serum.

The distribution of PGDH immunostaining is generally consistent with the biochemical distribution of PGDH activity in the fetal membranes.[4] The enzyme is absent from amnion, predominant in chorion, and present at low activities in decidua.[4, 17] The finding that trophoblast invading into decidua stained for PGDH raises the possibility that these cells are responsible for the PGDH activity found in dispersed cell preparations of decidua that were prepared by scraping it from the chorion. Clearly, separation of chorion and decidua cannot be accomplished without contamination by this simple mechanical procedure, and reports of decidual PGDH activity[17] must be regarded with some suspicion.

Our results suggest that the high PGDH activity present in the placenta[6, 7, 18] may be localized to intermediate trophoblast cells, and to syncytiotrophoblast, but not to cytotrophoblast or villous stromal tissue. It has been suggested that human placental PGDH is partially dependent on fetal genotype[19] and may be inhibited by progesterone,[20] as in the fetal lung,[21] or by estrogen.[20] An endogenous inhibitor of placental PGDH has also been reported,[22] but there is lack of change in PGDH activity with labor,[23, 24] which suggests that it is not a major regulatory factor contributing to the levels of bioactive primary prostaglandins. We did not observe differences in the localization of immunoactive PGDH in placenta or membranes with labor. Thus our results would be consistent with the view that changes in PGDH distribution or activity are unlikely to contribute to the increase in prostaglandin concentration at parturition. Prostaglandin H_2 synthase has also been localized primarily to syncytiotrophoblast and intermediate trophoblast, and similarly, its distribution did not change with labor.[25] However, placental PGDH could influence the amount of primary prostaglandins produced within the placenta that reaches the underlying decidua, myometrium, or both.

In late human pregnancy, amnion is a major site of prostaglandin E_2 production.[1-5] It has been suggested that primary prostaglandins may cross the fetal membranes from amnion to decidua or myometrium for subsequent biologic effects.[8, 9] Because of the high metabolic activity in chorion it has been suggested and subsequently shown[9] that this transfer likely takes place via the intercellular spaces. However, this study shows that PGDH is distributed heterogeneously in chorion. Thus we suggest that the chorion may not present the uniform metabolic barrier to prostaglandin transfer that previously had been assumed.

REFERENCES

1. Mitchell MD. The mechanism(s) of human parturition. J Dev Physiol 1984;6:107-18.
2. Novy MJ, Liggins GC. Role of prostaglandins, and prostacyclin, and thromboxanes in the physiologic control of the uterus and parturition. Semin Perinatal 1980;4:14-65.
3. Mitchell MD, Bibby JG, Hicks BR, Turnbull AC. Specific production of prostaglandin E_2 by human amnion in vitro. Prostaglandins 1978;15:377-82.
4. Okazaki T, Casey ML, Okita J, MacDonald PC, Johnston JM. Initiation of parturition XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. Am J Obstet Gynecol 1981;139:373-81.
5. Challis JRG, Olson DM. Parturition. In: Knobil E, Neill J, eds. The physiology of reproduction. New York: Raven Press, 1988:2177-2216.
6. Schlegel W, Greep RO. Prostaglandin 15-hydroxy dehydrogenase from human placenta. Eur J Biochem 1975; 56:245-52.
7. Jarabak J, Watkins JP. Purification of the human placental NAD^+-linked 15-hydroxy prostaglandin dehydrogenase. prostaglandins 1988;36:205-13.
8. Nakla S, Skinner K, Mitchell MD, Challis JRG. Changes in prostaglandin transfer across human fetal membranes obtained after spontaneous labor. Am J Obstet Gynecol 1986;155:1337-41.
9. Bennett PR, Chamberlain GVP, Myatt L, Patel L, Elder MG. Mechanisms of parturition: the transfer of prostaglandins across fetal membranes. In: Jones CT, ed. Research in perinatal medicine: fetal and neonatal development. Ithaca, New York: Perinatology Press, 1988: 407-9.
10. Yeh IT, O'Connor DM, Kurman RJ. Vacuolated cytotrophoblast: a subpopulation of trophoblast in the chorion laeve. Placenta 1989;10:429-38.
11. Hodsman AB, Steer BM, Arsenault AL. Aluminum intoxication in vitamine-D deficient rats; studies of bone aluminum localization and histomorphometry before and after vitamine-D repletion. J Bone Miner Res 1988;3:375-83.
12. Tai CL, Mak OT, Arai T, Tai HH. Monoclonal antibodies that inhibit the enzyme activity of NAD^+ dependent 15-hydroxy prostaglandin dehydrogenase. Biochem J 1990; 267:75-8.
13. Bourne G. The structure of the chorion. In: Human amnion and chorion. London: Lloyd-Luke, 1962:9-11.
14. Kurman RJ, Main CS, Chen HC. Intermediate trophoblast: a distinct form of trophoblast with specific morphological, biochemical, and functional features. Placenta 1984;5:349-70.
15. Gosseye S, Fox H. An immunohistochemical comparison of the secretory capacity of villous and extavillous trophoblast in the human placenta. Placenta 1984;5:329-48.
16. Khong TY, Lane EB, Robertson WB. An immunocytochemical study of fetal cells at the maternal-placental in-

terface using monoclonal antibodies to keratin, vimentin, and desmin. Cell Tissue Res 1986;246:189-95.
17. Cheung PY, Challis JRG. Prostaglandin E_2 metabolism in the human fetal membranes. Am J Obstet Gynecol 1989;161:1580-5.
18. Keirse MJNC, Hicks BR, Turnbull AC. Prostaglandin dehydrogenase in the placenta before and after the onset of labour. Br J Obstet Gynaecol 1976;83:152-5.
19. Erwich JJHM, Keirse MJNC. Human placental 15-hydroxy prostaglandin dehydrogenase activity is under fetal genetic control. Prostaglandins 1988;35:123-31.
20. Schlegel W, Remers LM, Hildebrandt-Stark HE, Behrman HR, Greep RO. Partial purification of human placental 15-hydroxy prostaglandin dehydrogenase: kinetic properties. Prostaglandins 1974;5:417-33.
21. Sun FF, Armour SB. Prostaglandin 15-hydroxy dehydrogenase and delta-13 reductase levels in the lungs of maternal, fetal, and neonatal rabbits. Prostaglandins 1974;7:327-38.
22. Nagai K, Mibe M, Mori N. Endogenous inhibitor of 15-hydroxy prostaglandin dehydrogenase in human placenta. Prostaglandins Leukotrienes Essen Fatty Acids 1989;38:157-61.
23. Eriwch JJHM, Keirse MJNC. Species differences in placental 15-hydroxy prostaglandin dehydrogenase [Abstract 123]. In: Proceedings of the First International Congress on Prostaglandins in Reproduction. Vienna, Austria, July 6-9, 1988.
24. Hansen HS. 15-hydroxy prostaglandin dehydrogenase. a review. Prostaglandins 1976;12:647-79.
25. Price TM, Kauma SW, Curry TE Jr, Clark MR. Immunohistochemical localization of prostaglandin endoperoxide synthase in human fetal membranes and decidua. Biol Reprod 1989;41:701-5.

The effect of physical activity during pregnancy on preterm delivery and birth weight

Mark A. Klebanoff, MD, MPH,[a] Patricia H. Shiono, PhD,[b] and J. Christopher Carey, MD,[c]

Bethesda, Maryland, Los Altos, California, and Oklahoma City, Oklahoma

The relationship between physical activity during pregnancy, preterm birth, and gestational age–adjusted birth weight was investigated prospectively in a cohort of 7101 women. This study is one of few to evaluate both employment- and non-employment-related physical activity. Prolonged periods of standing were associated with a modestly increased risk of preterm delivery (adjusted odds ratio for ≥8 hours/day of standing = 1.31). Heavy work or exercise was not associated with preterm delivery (adjusted odds ratio for ≥4 hours per day of heavy work = 1.04). The proportion of infants born preterm did not differ among women working in predominantly standing, active, and sedentary occupations. Physical activity was not associated with gestational age-adjusted birth weight after controlling for confounding variables. These data suggest that unmeasured socioeconomic differences among women reporting different levels of activity may account for previously described associations between physical activity and pregnancy outcome. Most pregnant women who report increased levels of physical activity are not at increased risk of preterm delivery or reduced intrauterine growth. However, these data do not address the role of activity restriction in the management of selected women at high risk for adverse pregnancy outcome. (Am J Obstet Gynecol 1990;163:1450-6.)

Key words: Infant, low birth weight; labor, premature; women, working; work

The effect of physical activity during pregnancy on preterm delivery and fetal growth has been controversial. Mamelle et al.[1] stated that as the number of fatigue factors among women employed during pregnancy increased from 0 to 5, the rate of premature birth increased from 2.3% to 11.1%. Among hospital workers, work-related factors (long periods of standing, carrying heavy loads, and performing heavy cleaning tasks) were observed to be associated with preterm delivery.[2] Compared with women whose occupations entailed varied amounts of physical activity, women employed in occupations requiring long periods of standing were found to be 2.7 times as likely to be delivered before term, and women employed in sedentary occupations were 1.4 times as likely to be delivered preterm.[3] In contrast, other studies have found the usual range of physical activities performed by pregnant women to be unassociated with gestational age–adjusted birth weight,[4] mean gestational length,[4] or preterm delivery.[5] However, only two studies considered non-employment-related physical activity.[4, 5] This study determines the relationship between physical activity and pregnancy outcome in a prospective cohort of women.

From the Division of Prevention Research, National Institute of Child Health and Human Development, National Institutes of Health,[a] the Center for the Future of Children, David and Lucile Packard Foundation,[b] and the Department of Obstetrics and Gynecology, University of Oklahoma. The authors are members of the VIP Study Group. Members of the group and their affiliations are listed at the end of the article.

Supported by a contract from the National Institute of Child Health and Human Development and the National Institute of Allergy and Infectious Diseases.

Presented before the Annual Meeting of the Society for Epidemiologic Research, Snowbird, Utah, June 13-15, 1990.

Received for publication May 8, 1990; revised June 29, 1990; accepted July 16, 1990.

Reprint requests: Mark A. Klebanoff, MD, MPH, Division of Prevention Research, National Institute of Child Health and Human Development, National Institutes of Health, EPN 640, Bethesda, MD 20892.

6/1/23815

Material and methods

The Vaginal Infections and Prematurity (VIP) study is a prospective study, sponsored by the National Institute of Child Health and Human Development and the National Institute of Allergy and Infectious Diseases, to determine the association between genital tract carriage of various microorganisms and preterm birth. The population consisted of women who received prenatal care at one of five clinical centers (Columbia University, New York; University of Washington, Seattle; University of Oklahoma, Oklahoma City; University of Texas, San Antonio; Louisiana State University, New Orleans) from 1984 to 1987.

Women who were at least 16 years old and did not have one of the exclusion criteria (diabetes requiring insulin, hypertension or heart disease requiring medication, chronic renal disease, multiple pregnancy, Rh sensitization, current use of corticosteroids, history of

Table I. Characteristics of the study population at 23 to 26 weeks' gestation

Characteristic	*n*	*% of Total*	*Characteristic*	*n*	*% of Total*
Site			Income (thousands of dollars)		
Columbia	2184	30.8	<5	2105	29.6
Seattle	491	6.9	5-9.999	1650	23.2
Oklahoma	2040	28.7	10-14.999	989	13.9
Texas	1105	15.6	15-19.999	291	4.1
New Orleans	1281	18.0	20-24.999	131	1.8
Ethnicity			25-29.999	72	1.0
White	2385	33.6	≥30	287	4.0
Hispanic	2476	34.9	Unknown	1576	22.2
Black	2240	31.6	Current smoking		
Age at interview (yr)			No	5204	73.3
<20	1679	23.6	Yes	1897	26.7
20-24	2493	35.1	Alcohol within past 3 mo		
25-29	1703	24.0	None	5413	76.2
≥30	1223	17.2	Any	1687	23.8
Education (yr)			Currently employed		
<12	3965	43.3	No	5321	75.0
12	2602	36.6	Yes	1778	25.0
>12	1423	20.0	Hours/day standing		
Marital status			0	3093	43.6
Married	3396	47.8	1-3	1644	23.2
Never married	2603	36.7	4-7	1466	20.6
Other	1097	15.4	≥8	898	12.6
Payment status			Hours/day doing heavy work		
Insurance	1229	17.5	0	5610	79.0
Medicaid	3147	44.8	1-3	1018	14.3
Self-pay	1941	27.6	≥4	472	6.6
Other	710	10.1	Hours/day doing light work		
Parity			0	173	2.4
0	2836	39.9	1-3	2878	40.5
1+	4265	60.1	4-7	2293	32.3
			≥8	1757	24.7

cervical incompetence or cerclage, receipt of antibiotics within the 2 weeks preceding interview, previous treatment with tocolytic agents between the beginning of the pregnancy and the date of interview, and intent to deliver at a non–study hospital) were interviewed at 23 to 26 completed weeks' gestation about a variety of risk factors for adverse pregnancy outcome. The interview included the question "In a typical day during the first 5 months of pregnancy, about how many hours did you spend doing the following?" Activities included "standing for long periods of time, heavy work/exercise" (sufficient to cause sweating), and "light work/exercise." These activities were asked with reference to the entire day, as opposed to on the job only. For these analyses, times were classified a priori as 0, 1 to 3, 4 to 7, and ≥8 hours per day. Few women reported engaging in heavy work for ≥8 hours per day; therefore the two highest categories were combined for heavy work.

Gestational age at screening for study eligibility (23 to 26 weeks) was determined according to the attending obstetrician's best estimate. This estimate was based on the last menstrual period, supplemented by other clinical evidence including ultrasonograms, uterine measurements, and detection of fetal heart tones. Gestation at delivery (in completed weeks) was calculated from this initial estimate and the elapsed time from screening to delivery.

The associations between risk factors for adverse outcome and activity level were evaluated by χ^2 analysis. The relative risk of preterm delivery by level of activity was approximated by odds ratios[6]; 95% confidence intervals was calculated with Woolf's method.[7] The odds ratios for preterm delivery were adjusted for the confounding variables listed in Table I by means of multiple logistic regression.[6] Because many hours of one type of activity might exert their effect by precluding the possibility of another activity, the final regression model contained terms for all types of work; results from this model were nearly identical to those obtained from three separate models, each limited to one type of activity.

The association between preterm birth and occupational characteristics was investigated to replicate the analysis of Teitelman et al.[3] Each woman's current occupation was classified according to the United States Census Bureau 1980 codes.[8] Only women whose job titles were identical to those used previously[3] were included. The risk of preterm birth was compared among women whose jobs were considered predominantly standing, active, or sedentary by this classification.

Finally, we studied the effect of reported physical activity on fetal growth. Mean gestational age–adjusted birth weight was calculated by means of a two-way analysis of variance for each level of reported physical activity. These mean birth weights were further adjusted for confounding factors using analysis of covariance.

Results

From Nov. 1, 1984, to June 30, 1987, 8287 women were enrolled in the VIP study; data on the duration of pregnancy were available for 7578 women. Missing data were caused by loss of the woman to follow-up between study entry and delivery. Extensive efforts were made to locate study women and obtain at least the date of delivery and birth weight and sex of the infant. These efforts, which were unsuccessful for 709 women, included attempted contact at the last known address, querying other hospitals in the region, and in many cases attempting to locate the birth certificate at the county health department. Early in the study, the questions regarding physical activity were revised; the original questions were not comparable with the revised ones, and women whose data were contained on these early forms were deleted (n = 256). Women whose ethnicity was other than white, black, or Hispanic were excluded (n = 207), as were women with no data on amount of physical activity (14 for standing, 14 for light work, 15 for heavy work). The final population comprised 7101 women for the study of standing and light work, and 7100 for heavy work. The characteristics of the study population are presented in Table I.

Women who reported long hours of heavy work had demographic profiles similar to those who reported long hours of standing.* Prolonged heavy work and standing were both more common among women at the San Antonio center, among Hispanic women, and among parous women. Women with increasing education, women under 20 years of age, women who were never married, and women who currently smoked were more likely to report no heavy work or prolonged standing. Longer hours of heavy work and standing were most prevalent among women who might be called the "working poor": currently employed, total household incomes of $10,000 to 14,999 per year, currently married or married at some time in the past, and self-payment for medical care.

Women who spent more time doing light work differed in these characteristics from women doing heavy work and standing. Increasing time spent doing light work was most prevalent among women in New York City and Seattle, white women, women aged 25 to 29 years, currently married women, and parous women. Longer hours of light work were more prevalent among women of higher socioeconomic status than were longer hours of heavy work and standing. Higher socioeconomic status was reflected in household incomes of ≥$20,000, currently working, more than 12 years of education, and having private medical insurance.

The relationships among preterm birth and hours spent in standing, heavy work/exercise, and light work/exercise were generally modest (Table II). In the unadjusted analysis women who spent more time standing had a modestly increased risk of preterm birth. Women who spent ≥8 hours/day standing were approximately 28% more likely to give birth before 37 completed weeks' gestation than women who reported no standing. Women who performed ≥4 hours/day of heavy work were 20% more likely to be delivered preterm than women who did no heavy work. Women who engaged in light work were less likely to be delivered before term. To determine whether local differences in physical activity accounted for the results, the associations between reported activity and preterm delivery were evaluated separately for each center. In no center were the results substantially different* from those in Table II.

The adjusted relationships between various risk factors and preterm delivery are presented in Table III. Preterm delivery was significantly more common among black women (odds ratio compared with white women = 1.28; 95% confidence interval = 1.01 to 1.63), women who smoked at the time of interview (odds ratio compared with nonsmokers = 1.47; 1.23 to 1.75), and women in Texas (odds ratio compared with Columbia = 1.51; 1.11 to 2.05). The relationship between household income and preterm delivery was complex; preterm delivery decreased with increasing income up to $15,000 to 19,999 per year (odds ratio compared with <$5000 = 0.36), then rose again to reach values similar to those for women in the lowest category (odds ratio for ≥$30,000 = 0.90). The other factors in Table III were not significantly associated with preterm birth.

Long hours of standing remained significantly associated with preterm birth, although the effect was modest (adjusted odds ratio = 1.31; 1.01 to 1.71). The trend for increasing preterm birth with increasing time standing was of borderline statistical significance. Heavy work was not associated with preterm birth. However, increasing time spent doing light work or exercise retained its significant negative association with preterm birth.

Among the VIP study population, 1778 women (25% of the total) were working at 23 to 26 weeks' gestation; 1252 (70%) were employed in jobs with titles that were

*Detailed characteristics are available on request from the authors.

Table II. Preterm delivery by activity level

Activity	*Percent preterm*	*Odds ratio*	*95% Confidence interval*
Standing			
0 hr	10.2	1.00	—
1-3 hr	12.6	1.27	1.05-1.53
4-7 hr	11.7	1.17	0.96-1.42
≥8 hr	12.7	1.28	1.02-1.61
		p for trend = 0.046	
Heavy work			
0 hr	11.2	1.00	—
1-3 hr	11.6	1.04	0.84-1.28
≥4 hr	13.1	1.20	0.91-1.59
		p for trend = 0.216	
Light work			
0 hr	16.2	1.00	—
1-3 hr	12.1	0.71	0.47-1.09
4-7 hr	11.3	0.66	0.43-1.01
≥8 hr	9.8	0.56	0.36-0.87
		p for trend = 0.005	

Table III. Factors associated with preterm delivery after adjustment*

Characteristic	*Odds ratio*	*Characteristic*	*Odds ratio*
Site		Income (thousands of dollars)	
Columbia	1.00†	<5	1.00†
Seattle	1.01	5-9.999	0.82
Oklahoma	1.17	10-14.999	0.73‡
Texas	1.51‡	15-19.999	0.36‡
New Orleans	0.99	20-24.999	1.05
		25-29.999	1.70
		≥30	0.90
		Unknown	0.95
Ethnicity		Current smoking	
White	1.00†	No	1.00†
Hispanic	1.07	Yes	1.46‡
Black	1.28‡		
Age at interview (yr)		Alcohol within past 3 mo	
<20	1.10	None	1.00†
20-24	1.08	Any	0.96
25-29	1.00†		
≥30	1.23		
Education (yr)		Parity	
<12	0.96	0	1.00†
12	1.00†	1†	1.01
>12	0.90		
Marital status		Currently employed	
Married	1.00†	No	1.00†
Never married	1.10	Yes	1.02
Other	1.01		
Payment status			
Insurance	1.00†		
Medicaid	1.01		
Self-pay	0.92		
Other	1.03		

*Adjusted for the factors listed in the table, plus time spent standing and doing heavy and light work.
†Reference group.
‡$p < 0.05$ versus reference group.

classifiable according to the categories of Teitelman et al.[3] This is similar to the 63% of employed women in the New Haven population with classifiable jobs.[3] Among those in "active" jobs (n = 551), 10.5% were delivered preterm; among those in "standing" jobs (n = 435), 11.0% were delivered preterm; among those in "sedentary" jobs (n = 266), 8.2% were delivered preterm. None of these percentages was significantly dif-

Table IV. Adjusted odds ratios for preterm delivery and adjusted mean birth weight by activity level

	Adjusted odds ratio for preterm delivery*	*95% Confidence interval*	*Adjusted† mean birth weight (gm)*
Standing			
0 hr	1.00	—	3230
1-3 hr	1.26	1.03-1.53	3229
4-7 hr	1.19	0.95-1.48	3219
≥8 hr	1.31	1.01-1.71	3198
	p for trend = 0.061		p for trend = 0.120
Heavy work			
0 hr	1.00	—	3210
1-3 hr	0.94	0.75-1.18	3187
≥4 hr	1.04	0.76-1.42	3261
	p for trend = 1.00		p for trend = 0.289
Light work			
0 hr	1.00	—	3182
1-3 hr	0.74	0.48-1.14	3250
4-7 hr	0.69	0.44-1.07	3217
≥8 hr	0.59	0.38-0.93	3226
	p for trend = 0.019		p for trend = 0.247

*Adjusted for study site, ethnicity, age, education, parity, marital status, income, smoking, alcohol consumption, insurance status, current employment.

†Adjusted for the above factors plus gestational age.

ferent from any of the others; adjustment for the factors in Table III had little effect on these results.

To determine the degree to which active, standing, and sedentary jobs were associated with reported activity, the mean hours of standing, light work, and heavy work were compared for women employed in the three types of jobs. Women employed in active, standing, and sedentary jobs reported 4.5, 5.6, and 1.7 hours of prolonged standing ($p < 0.0001$ by analysis of variance); 1.1, 0.7, and 0.3 hours of heavy work ($p < 0.0001$); and 6.1, 5.2, and 5.7 hours of light work ($p = 0.0002$). These data indicate that although the job classifications are somewhat associated with reported activity, the associations are not strong, possibly because they fail to consider nonoccupational physical activity.

To investigate the possibility that errors in calculation of gestational age contributed to the inability to find a strong association of preterm birth and level of strenuous activity, the relationship between activity and low birth weight (<2500 gm) was investigated. The percentage of infants with low birth weights among women reporting 0, 1 to 3, 4 to 7, and ≥8 hours of standing were 7.3%, 7.5%, 7.8%, and 7.6%, respectively. The corresponding figures for light work were 12.4%, 7.3%, 7.8%, and 6.8%. Among women reporting 0, 1 to 3, or ≥4 hours per day of heavy work, the low birth weight percentages were 7.5%, 7.5%, and 7.3%, respectively.

Activity might have a greater effect on fetal growth than on length of pregnancy. To investigate this possibility, we calculated the mean gestational age–adjusted birth weight for infants of women in each activity group. Mean gestational age–adjusted birth weight did not vary significantly with reported hours of standing or heavy work (p values for trend 0.48 and 0.22, respectively). Increasing time spent doing light work was associated with increased gestational age–adjusted birth weight in the unadjusted analysis, increasing from 3181 gm for women reporting 0 hours of light work to 3300 gm for women reporting ≥8 hours of light work (p for trend = 0.048). After adjustment for the factors listed in Table I, standing, heavy work, and light work were not associated with gestational age–adjusted birth weight (Table IV).

Comment

This study found that women who reported long hours of standing had a slightly increased risk of preterm delivery. Heavy work was not associated with an increased risk of preterm delivery, and light work demonstrated a negative association with preterm delivery. These findings are in general agreement with those of Berkowitz,[9] who noted that an absence of leisure time physical activity was a risk factor for preterm birth, and who did not find a wide variety of measures of physical activity to be associated with preterm birth.[5] Occupational activity was not related to mean birth weight or gestational age in other studies.[10]

These results disagree with those of previous studies[1-3] that found occupational factors, including prolonged standing, to be strongly associated with preterm birth. Both our study and that of Teitelman et al.[3] were prospective; activity data were collected before delivery. The other reports were based on retrospectively ascertained data.[1, 2] Our study differs from the former studies[1-3] in one important respect: data on activity were not limited to occupational factors, but included all physical activity. However, when only occupational factors were considered, no difference in preterm delivery was found among women employed in standing, active, and sedentary jobs. We obtained occupational data at the end of the second trimester, when physical activity would be expected to exert its greatest effect; Teitelman

et al.[3] obtained most occupational data during the first trimester. Differential rates of quitting work as pregnancy progressed were less likely to be of concern in our study.

There is no obvious reason to explain why we were unable to replicate the results of Teitelman et al.[3] and Mamelle et al.[1]; a possible explanation may be the socioeconomic difference between the study populations. The New Haven and French populations comprised a broad cross-section of women with widely varying occupations. In those settings, reported occupational physical activity may have been a surrogate for the more difficult to measure social class. Our predominantly clinic-based population demonstrated relatively little socioeconomic variability; 68% of women whose household income was known earned <$10,000 per year and only 17% had private health insurance. This relative socioeconomic homogeneity may account for the minimal effect of physical activity, both occupational and total, on preterm birth. Alternatively, the generally high-risk nature of the VIP population, with a preterm delivery rate of approximately 10%, may have obscured a subtle effect of physical activity on preterm birth.

It could be argued that women employed in jobs that required prolonged standing were more likely to quit work as pregnancy advanced, thereby reducing their risk of adverse outcome. The VIP protocol called for random samples of women with uncomplicated pregnancies to undergo an abbreviated reinterview at 31 to 33 and 34 to 36 weeks. Of the 1252 women employed in classifiable occupations[3] at 23 to 26 weeks, 156 were reinterviewed. Sixty-seven percent of women with jobs requiring prolonged standing were still working, compared with 55% of those with active and 78% of those with sedentary jobs. Therefore the absence of an increased risk of preterm delivery among women with jobs that require prolonged standing is not a result of their likelihood to quit work earlier in pregnancy than women with other jobs.

Reported physical activity was not associated with gestational age–adjusted birth weight. This is in agreement with Rabkin et al.,[4] who collected extensive prospective data on total physical activity at 17, 28, and 36 weeks' gestation. They found no associations between activity at either paid work or house work at any of the three time points and birth-weight-for-gestational age. Our results are also similar to those of Zuckerman et al.,[10] who found no difference in mean birth weight among women employed in standing occupations, other occupations, and unemployed women.

In summary, we studied the effect of physical activity on the subsequent development of preterm birth and on gestational age–adjusted birth weight. Women engaged in prolonged periods of standing were at slightly increased risk of preterm delivery; heavy work or exercise was not associated with preterm delivery. Women who engaged in light work or exercise during the first 5 months of pregnancy were at reduced risk of preterm delivery. The percentage of infants born before term did not differ among women engaged in predominantly standing, active, and sedentary occupations. Gestational age–adjusted birth weight was not associated with reported hours of standing, heavy work, or light work. These results cast doubt on the association between physical activity of the type usually practiced by pregnant women and either duration of pregnancy or gestational age–adjusted birth weight, and raise the possibility that unmeasured socioeconomic differences between women reporting different levels of activity may account for previous positive findings on this subject.

Although little risk was observed for physical activity and preterm birth, there may be a place for activity restriction among women who experience pregnancy complications. However, activity restriction may have unanticipated adverse consequences. Employed women may face a dilemma: they may be forced to work to retain pay and medical benefits, thereby risking the pregnancy, or they may quit working and lose pay, benefits, and perhaps the job. Women with other children or household responsibilities may be unable to restrict their activity despite the possible threat to their pregnancy. Unfortunately, these issues are not easily resolved.

The VIP study group consists of the following:

Sumner J. Yaffe, MD, Charlotte S. Catz, MD, Robert P. Nugent, PhD, George G. Rhoads, MD, MPH (now at the University of Medicine and Dentistry of New Jersey), Donald McNellis, MD, Mark A. Klebanoff, MD, MPH, Howard J. Hoffman, MA, Heinz W. Berendes, MD, MHS, National Institute of Child Health and Human Development

Robert Edelman, MD (now at the University of Maryland), William C. Blackwelder, PhD, Richard A. Kaslow, MD, MPH, George F. Reed, PhD, Mary Frances Cotch, PhD, National Institute of Allergy and Infectious Diseases

Joan A. Regan, MD, Columbia University; Sterling Williams, MD, Harlem Hospital Center

David H. Martin, MD, Joseph Pastorek, MD, Louisiana State University, New Orleans; Paul Sommers, MD, Tulane University

J. Christopher Carey, MD, Philip J. Rettig, MD, University of Oklahoma, Oklahoma City

Ronald S. Gibbs, MD (now at the University of Colorado) University of Texas, San Antonio

David A. Eschenbach, MD University of Washington, Seattle

A. Vijaya Rao, PhD, W. Kenneth Poole, PhD, Research Triangle Institute, Research Triangle Park, North Carolina

REFERENCES

1. Mamelle N, Laumon B, Lazar P. Prematurity and occupational activity during pregnancy. Am J Epidemiol 1984; 119:309-22.

2. Saurel-Cubizolles MJ, Kaminski M, Llado-Arkhipoff J, et al. Pregnancy and its outcome among hospital personnel according to occupation and working habits. J Epidemiol Community Health 1985;39:128-34.
3. Teitelman AM, Welch LS, Hillenbrand KG, Bracken MB. Effect of maternal work activity on preterm birth and low birth weight. Am J Epidemiol 1990;131:104-13.
4. Rabkin CS, Anderson HR, Bland JM, Brooke OG, Chamberlain G, Peacock JL. Maternal activity and birth weight: a prospective population-based study. Am J Epidemiol 1990;131:522-31.
5. Berkowitz GS, Kelsey JL, Holford TR, Berkowitz RL. Physical activity and the risk of spontaneous premature delivery. J Reprod Med 1983;28:581-8.
6. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic research. Belmont, California: Lifetime Learning Publications, 1982:140-58, 419-46.
7. Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955;19:251-3.
8. U.S. Department of Commerce, Bureau of the Census. 1980 census of population alphabetical index of industries and occupations. Publication no. PHC80-R3, November 1982.
9. Berkowitz GS. An epidemiologic study of preterm delivery. Am J Epidemiol 1981;113:81-92.
10. Zuckerman BS, Frank DA, Hingson R, Morelock S, Kayne HL. Impact of maternal work outside the home during pregnancy on neonatal outcome. Pediatrics 1986;77:459-64.

Prenatal diagnosis of laryngeal atresia

William J. Watson, MD, John M. Thorp, Jr., MD, Richard C. Miller, MD, Nancy C. Chescheir, MD, Vern L. Katz, MD, and John W. Seeds, MD

Chapel Hill, North Carolina

In a patient in whom preeclampsia developed at 23 weeks' gestation, ultrasonographic examination of the fetus showed enlarged edematous lungs, a compressed fetal heart, severe ascites, fetal hydrops, and placental edema. Autopsy of the hydropic stillborn infant showed laryngeal atresia. The ultrasonographic appearance of this rare malformation is presented. (Am J Obstet Gynecol 1990;163:1456-7.)

Key words: Laryngeal atresia, prenatal diagnosis, hydrops fetalis

Laryngeal atresia is a rare congenital malformation that is incompatible with life. Antenatal diagnosis of this malformation is possible because of the secondary structural changes in the fetus that result from this condition. This report details the ultrasonographic findings and prenatal course of a patient whose fetus had laryngeal atresia.

Case report

A 34-year-old woman, gravida 3, para 1-0-1-1, was first seen for prenatal care at 7 weeks' gestation. At 15 weeks' gestation an ultrasonographic examination showed normal fetal anatomy and the patient chose to have an amniocentesis because of increased maternal age. Amniotic fluid α-fetoprotein was 0.8 multiples of the median, and the karyotype was 46,XX. At 23 weeks' gestation, the patient's blood pressure was 146/90 mm Hg and she had gained 8 pounds in 1 week. Repeat ultrasonographic examination showed a hydropic fetus with massively enlarged lungs, a compressed heart that appeared structurally normal (Fig. 1), severe ascites, and scalp edema. The placenta was thick and echogenic, and the amniotic fluid volume was decreased. Significant laboratory values included a uric acid level of 7.1 gm/dl and aspartate aminotransferase of 32 U/L (normal, 9 to 26 U/L). There was no proteinuria. Laboratory evaluation for hydrops included a negative antibody screen, and negative toxoplasmosis, rubella, cytomegalovirus, herpes simplex, and parvovirus titers.

A diagnosis of preeclampsia was made and induction of labor was accomplished for maternal indications with prostaglandin E_2 suppositories. A severely hydropic stillborn 1650 gm female fetus was delivered. At autopsy, laryngeal atresia was found. The fetal lung was hyperplastic and weighed 70 gm (normal, 15 gm). There was massive ascites, with 1000 ml of peritoneal fluid. Duodenal atresia was also noted. The placenta weighed 560 gm and showed hydropic change.

From the Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill.
Received for publication June 15, 1990; accepted June 22, 1990.
Reprint requests: William J. Watson, MD, Department of Obstetrics and Gynecology, Maternal Fetal Medicine Division, University of North Carolina at Chapel Hill, CB 7570 MacNider, Chapel Hill, NC 27599-7570.
6/1/23355



Fig. 1. Fetal chest at level of four-chamber view. Massive enlargement of fetal lung is seen, causing compression of heart.

Comment

The most striking ultrasonographic finding in this fetus at 23 weeks' gestation was the enlarged echogenic fetal lung (Fig. 1). The hyperplastic lung results from fetal lung secretions that accumulate in the tracheobronchial tree. It has been hypothesized that excessive retention of liquid in the lung, as is seen with laryngeal atresia, promotes lung growth.[1] The fetal lung compression on the heart, great vessels, and thoracic duct may have caused the fetal hydrops.

We were able to find one previous report of the ultrasonographic appearance of laryngeal edema—in the Japanese literature.[2] This patient had a normal ultrasonographic examination at 18 weeks, but at 22 weeks' gestation ascites and enlarged echogenic lung were found. She eventually was delivered of a 2578 gm infant who died shortly after birth after failed attempts at intubation.

Factors that influence amniotic fluid volume in this condition may include the presence of other associated anomalies and the degree of esophageal and stomach compression from ascitic fluid.

Included in the differential diagnosis of a fetus with enlarged echogenic lungs is type III congenital cystic adenomatoid malformation of the lung, which may also be associated with fetal hydrops. However, this lesion is usually unilateral.

Direct visualization of the fetal larynx with ultrasonography to diagnose atresia is probably not possible at present, but the secondary structural changes in the fetal chest that result from this rare lesion are distinctive and may make antenatal diagnosis of this malformation possible. Ultrasonographic recognition of these characteristic changes may not be possible until after the middle of the second trimester because ultrasonography earlier in pregnancy showed normal fetal anatomy.

REFERENCES

1. Wigglesworth JS, Desai R, Hislop AA. Fetal lung growth in congenital laryngeal atresia. Pediatr Pathol 1987;7:515-25.
2. Arizawa M, Imai S, Suehara N, Nakayama M. Prenatal diagnosis of laryngeal atresia [Translation]. Nippon Sanka Fijinka Gakki Zasshi 1989;41:907-10.

Correlations between antepartum maternal metabolism and newborn behavior

Thomas Rizzo, PhD,[c, d] Norbert Freinkel, MD,[a, b, d, †] Boyd E. Metzger, MD,[a, d] Roger Hatcher, PhD,[c, d] William J. Burns, PhD,[c, d] and Peter Barglow, MD[c, d]

Chicago, Illinois

We suggested that antepartum maternal metabolism may affect later cognitive and behavioral function of progeny by impacting on developing brain cells in utero. This study reports on the observed relationships between serial characterizations of maternal fuels during pregnancy and Brazelton neonatal behavioral assessment scale ratings of offspring from 73 well-controlled pregestational diabetic patients, 112 gestational diabetic patients, and 24 nondiabetic patients. After controlling for the effects of premature birth on the Brazelton neonatal behavioral assessment scale, significant correlations were found between second- and third-trimester glycemic regulation (hemoglobin A_{1c} and fasting plasma glucose levels) and three of four newborn behavioral dimensions of the Brazelton neonatal behavioral assessment scale. In each case, as maternal glucose increased, the newborns' Brazelton neonatal behavioral assessment scale responses were poor. Results were not substantially different when gestational and pregestational diabetic patients were analyzed separately, nor can they be attributed to various perinatal events (neonatal asphyxia, hypoglycemia) or differences in socioeconomic status or ethnicity. The presence of fuel-related neurobehavioral deficits in neonates of diabetic mothers suggests that such infants start their interactions with care givers from a modified base. (Am J Obstet Gynecol 1990;163:1458-64.)

Key words: Behavioral teratogenesis, fuel-mediated teratogenesis, offspring of diabetic mothers, newborn behavior

Most maternal fuels gain access to the developing fetus in a concentration-dependent fashion throughout pregnancy.[1] As a consequence, fuel metabolism in the mother may determine the quantitative and qualitative characteristics of the nutrient mixture in which the fetus develops in utero.[1, 2] This tissue culture aspect of pregnancy prompted the suggestion that maternal fuel economy may influence long-range characteristics of the progeny by modifying the phenotypic gene expression of poorly replicating or terminally differentiated cells during embryogenesis or fetal development.[3, 4] Freinkel[3, 4] designated the developmental possibilities as "fuel-mediated teratogenesis" and hypothesized that the long-range manifestations would depend on the cells in the fetus undergoing differentiation, proliferation, or functional maturation at the time of the disturbance in maternal fuels.

From the Departments of Medicine,[a] Molecular Biology,[b] and Psychiatry[c] and the Center for Endocrinology, Metabolism, and Nutrition,[d] Northwestern University Medical School and Northwestern Memorial Hospital (Prentice Women's Hospital).
Supported in part by National Institutes of Health awards DK10699, DK07169, HD11021, HD19070, HD62903, and RR48.
Received for publication February 13, 1990; revised July 20, 1990; accepted July 27, 1990.
Reprint requests: Dr. Thomas Rizzo, Northwestern University, Searle Building 10-526, 303 E. Chicago Ave., Chicago, IL 60611.
†Deceased.
6/1/24150

We initiated a program at Northwestern University Medical School in 1977 to test for fuel-mediated teratogenesis on a prospective basis. Diabetes was chosen as the operational paradigm because maternal fuels undergo the greatest perturbation in this condition. Thus all women with pregnancies complicated by diabetes mellitus who were seen at Prentice Women's Hospital and Maternity Center of Northwestern Memorial Hospital between September 1977 and February 1983, and a smaller number of pregnant women with documented normal carbohydrate metabolism, were given the opportunity to participate in this prospective study. They were enrolled in the Northwestern University Diabetes in Pregnancy Center, and serial estimates of circulating maternal fuels were obtained throughout the antepartum management as a data base against which the project detailed examinations of the offspring at birth and during infancy and childhood.

All gravid women with diabetes were given diabetes care consistent with the prevailing state of the art including multiple insulin injections each day. Thus severe metabolic abnormalities were not encountered in any participant during the 1977 to 1983 period of enrollment, and a perinatal mortality rate of 1.9% was achieved even in the patients with insulin-dependent diabetes mellitus. Nevertheless, we have been able to show some distinct correlations between antepartum fuel metabolism and a number of subsequent charac-

teristics of the offspring[5] thereby attesting to the exquisite sensitivity of fuel-mediated teratogenesis.

This manuscript summarizes the relationship between maternal fasting plasma glucose, hemoglobin A_{1C} (HbA_{1C}), and β-hydroxybutyrate during the second and third trimesters of pregnancy and neonate performance on the Brazelton neonatal behavioral assessment scale.[6] Portions of this manuscript were reported previously in abstract form.[7]

Material and methods

Subjects. Subjects were 209 pregnant women and their singleton neonates. Seventy-three gravid women had known diabetes that antedated the pregnancy, that is, pregestational diabetes mellitus, 112 gravid women had "glucose intolerance with onset or first recognition during pregnancy"[8] that is, gestational diabetes mellitus, and 24 gravid women had normal gestational carbohydrate metabolism (nondiabetic patients) as documented by oral glucose tolerance according to the criteria of O'Sullivan and Mahan.[9] We excluded all women who required medication on a chronic basis for the treatment of medical or psychiatric problems or who were seriously noncompliant (i.e., repeatedly failed to complete scheduled tests, keep clinic appointments, or maintain written records of home monitoring for urinary glucose, acetone, or capillary blood sugar levels as this became available). All participants gave informed written consent, and detailed demographic and socioeconomic data were collected by interview with Diabetes in Pregnancy Center staff.

Management of mothers during pregnancy. Antepartum care was provided according to a detailed protocol by a team of diabetologists, obstetricians, neonatologists, nurse educators, dieticians, and social workers. The core of senior physician-investigators remained unchanged throughout the 66-month period of enrollment. All women were seen at the outpatient clinic at least biweekly before 30 weeks' gestation and weekly thereafter. Women with insulin-requiring diabetes were hospitalized for approximately 1 week immediately after enrollment to assess metabolic status and improve diabetes education. A second hospitalization for further improvement of diabetes regulation was instituted at 28 weeks' gestation for women who had enrolled before week 24.

At each outpatient visit and twice weekly during hospitalization, blood samples were drawn after overnight fast to measure plasma glucose[10] and β-hydroxybutyrate levels.[11] Monthly estimates of HbA_{1C} levels were obtained by column chromatography with macrocolumns.[12] Mean values for fasting plasma glucose, β-hydroxybutyrate, and HbA_{1C} during the second and the third trimesters were derived for each subject by averaging all measurements obtained during those intervals. Entry into the program was sufficiently early to secure adequate biochemical documentation of the second trimester in 88% of the women with pregestational diabetes, 53% of the women with gestational diabetes mellitus, and 75% of the women with normal glucoregulation.

Neonatal care. Research personnel attended all deliveries; infants were immediately transferred to Ohio bed warmers and Apgar scores were recorded at 1 and 5 minutes. Umbilical artery pH at delivery was measured in blood drawn from double-clamped segments of umbilical cord. Plasma glucose was determined in cord blood at delivery and in blood samples obtained by heel stick at hourly intervals for the first 4 hours thereafter. Initial feeding was at 4 hours after birth unless hypoglycemia (plasma glucose ≤30 mg/L) was encountered. In that case, infants were fed immediately or intravenous glucose was administered. Determinations of plasma calcium and bilirubin levels and continued assessments of plasma glucose level and vital signs were also obtained. Complete physical examinations and estimations of gestational age as per Dubowitz[13] and Lubchenco[14] were performed by senior neonatologists.

Brazelton neonatal behavioral assessment scale testing. Three of the authors (R. H., P. B., and W. B.) performed all Brazelton neonatal behavioral assessment scale testing. They had been trained to an interrater reliability of >90% at the Brazelton training facility at Children's Hospital in Boston before the inception of the study. Reliability checks were conducted once or twice per year to prevent examiner drift. All 209 infants were examined without knowledge of the mother's metabolic status in a temperature- and light-controlled room near the regular care nurseries. Examinations of preterm infants (i.e., <37 weeks' gestational age) were scheduled at 40 weeks' gestational age to correct for neurobehavior maturation effects. Term infants (37 to 41 weeks) were seen on the second or third day after birth before discharge from the hospital.

Individual Brazelton neonatal behavioral assessment scale responses were categorized into the following four dimensions as per Als, Tronick, Lester, and Brazelton[15]: interactive dimension; motoric dimension; state control dimension; and physiologic control dimension. Descriptions of each dimension and its constituent items are presented in Table I. The interactive, motoric, and state control dimensions were scored 0 = excellent, 1 = average, and 2 = deficient. The physiologic control dimension was scored 0 = average, 1 = deficient.

Statistics. Data were analyzed by the partial correlation, one-way analysis of variance, and multivariate analysis of variance programs provided in the SPSS-X statistical software package[16] χ^2 analyses, and the Fisher exact probability test. Specific group comparisons were

Table I. Brazelton neonatal behavioral assessment scale dimensional analysis*

Dimension	*Assessment*	*Constituent items*
Interactive	Infant's capacity for attention and social responsiveness	Visual and auditory orienting responses, alertness, cuddliness, and consolability
Motoric	Infant's neuromuscular organization and maturity	Motor tone, hand-to-mouth and pull-to-sit abilities, motor maturity, defensive reaction, general activity level, and 21 elicited reflexes
State control	Infant's capacity to control his/her state of consciousness	Habituation to repeated stimuli, peak of excitement, lability of states, rapidity of buildup, irritability, and self-quieting
Physiologic control	Infant's autonomic lability responsiveness	Startle responses, tremulousness, and lability of skin color

*Adapted from Als H, Tronick E, Lester B, Brazelton TB. Handbook of Infant Development. New York: Wiley, 1979.

made by partitioning 3 × 2 contingency tables as per Castellan[17] within the χ^2 analyses and with Tukey's multiple range test within the analysis of variance and multivariate analysis of variance. Comparisons of partial correlation results were performed according to the procedure of McNemar.[18]

Results

Maternal metabolism during pregnancy. On average, women with pregestational diabetes mellitus were enrolled at 13.2 (SEM = 0.09) weeks' gestation, women with gestational diabetes mellitus at 23.5 (SEM = 0.08) weeks, and nondiabetic study participants at 17.9 (SEM, 1.2 weeks). Values for maternal HbA_{1C}, fasting plasma glucose and β-hydroxybutyrate during the second and third trimesters (mean ± SEM and the range of observed values) for each patient group are summarized in Table II. Multivariate analyses of variance performed on the mean values for each metabolite by patient group (patients with pregestational diabetes mellitus, patients with gestational diabetes mellitus, and nondiabetic patients) and trimester (within subjects factor) showed*: (1) Mean levels of second- and third-trimester fasting plasma glucose and HbA_{1C} were greater in mothers with pregestational diabetes mellitus than those with gestational diabetes mellitus and nondiabetic patients (all $p < 0.05$) and in patients with gestational diabetes mellitus vs nondiabetic patients (all $p < 0.05$); (2) mean second- and third-trimester β-hydroxybutyrate values did not differ significantly between the two diabetic groups; however, both values were significantly higher in mothers with diabetes than in the nondiabetic group ($p < 0.05$); (3) mean fasting plasma glucose values were significantly higher in the second than in the third trimester for both diabetic groups ($p < 0.001$); (4) mean HbA_{1C} was higher in the second than in the third trimester only for the group with pregestational diabetes mellitus ($p < 0.05$); and (5) mean β-hydroxybutyrate was significantly higher in the third than in the second trimester for the group with gestational diabetes mellitus and the nondiabetic group ($p < 0.01$). Before enrollment in our Diabetes in Pregnancy Center, most subjects with pregestational diabetes mellitus were not in optimal control and those with gestational diabetes mellitus were untreated. This most likely accounts for higher mean values of fasting plasma glucose and HbA_{1C} in the second trimester than in the third trimester.

*Since the multivariate analysis of variance is designed to evaluate the effect of trimester on maternal metabolism, subjects missing a second- or third-trimester HbA_{1C}, fasting plasma glucose, or β-hydroxybutyrate value were excluded from the respective analysis. Consequently, the analyses presented here involved 113 of the 185 (61%) gravid women with HbA_{1C} values, 80 of the 127 (63%) gravid women with β-hydroxybutyrate values, and 141 of the 209 (67%) gravid women with fasting plasma glucose values. Analyses of variance performed on the means for each metabolite at each trimester determined that there were no significant differences between gravid patients included in the multivariate analyses of variance (i.e., with metabolic values in both trimesters) and those omitted from these analyses because of missing metabolic data in one of the two trimesters.

Physical findings in newborn. Pertinent neonatal and delivery information is summarized in Table III. One-way analyses of variance were performed on the mean values per patient group for gestational age of delivery, birth weight, and cord glucose. The χ^2 analyses were performed on the incidence of preterm deliveries (gestational age <37 weeks), neonatal macrosomia (birth weight >4000 gm), hypoglycemia, hypocalcemia, hyperbilirubinemia, and 1- and 5-minute Apgar scores <8. Differences in the incidence of neonatal asphyxia (cord pH <7.1) in patients with pregestational diabetes mellitus vs patients with gestational

Table II. Indexes of metabolic control

	Second trimester			*Third trimester*		
	Mean ± SEM	*Range*	*No.*	*Mean ± SEM*	*Range*	*No.*
Pregestational diabetes mellitus (n = 73)						
HbA_{1c} (%)	5.9 ± 0.2	3.6-9.1	52	5.6 ± 0.1	3.6-8.9	67
Fasting plasma glucose (mg/dl)	126 ± 5	70-231	64	104 ± 2	69-150	73
β-hydroxybutyrate (mmol/L)	0.18 ± 0.02	0.02-0.58	29	0.19 ± 0.02	0.06-0.60	34
Gestational diabetes mellitus (n = 112)						
HbA_{1c}	5.2 ± 0.1	3.5-7.8	47	5.2 ± 0.1	3.5-7.4	96
Fasting plasma glucose	104 ± 2	69-153	59	92 ± 1	68-136	112
β-hydroxybutyrate	0.15 ± 0.01	0.07-0.35	37	0.17 ± 0.01	0.03-0.40	72
Nondiabetic (n = 24)						
HbA_{1c}	4.7 ± 0.3	3.3-6.4	17	4.3 ± 0.1	3.3-5.6	22
Fasting plasma glucose	78 ± 1	64-88	18	75 ± 1	62-89	24
β-hydroxybutyrate	0.10 ± 0.01	.04 ± 0.19	16	0.12 ± 0.01	0.05-0.23	21

Mean levels of second- and third-trimester fasting plasma glucose and HbA_{1c} were greater in patients with pregestational diabetes mellitus than those with gestational diabetes mellitus and nondiabetic patients (all $p < 0.05$) and in patients with gestational diabetes mellitus vs nondiabetic patients (all $p < 0.05$). Patients with pregestational diabetes mellitus and gestational diabetes mellitus did not differ significantly in mean second- or third-trimester β-hydroxybutyrate; however, both groups had significantly higher β-hydroxybutyrate values than those of nondiabetic patients at both time points ($p < 0.05$). Mean HbA_{1c} was higher in the second than in the third trimester only for the group with pregestational diabetes mellitus ($p < 0.005$). Mean fasting plasma glucose was higher in the second than in the third trimester for patients with pregestational diabetes mellitus ($p < 0.001$) and gestational diabetes mellitus ($p < 0.001$), but not for nondiabetic patients. Mean β-hydroxybutyrate was higher in the third than in the second trimester for the group with gestational diabetes mellitus ($p < 0.05$) and the nondiabetic ($p < 0.01$) group only.

Table III. Status at delivery and neonatal morbidities

	Pregestational diabetes mellitus	*Gestational diabetes mellitus*	*Nondiabetic*
No. of subjects	73	112	24
Gestational age at delivery (wk)*	38.2 ± 0.2	38.6 ± 0.2	39.5 ± 0.4
Delivery at <37 wk gestational age (%)	10	14	8
Birth weight (gm)†	3644 ± 84	3554 ± 62	3243 ± 106
Macrosomia (%)	29	27	13
Cord glucose	115 ± 6	105 ± 4	97 ± 4
Cord pH<7.1 (%)	4	0	0
Hypoglycemia‡ (%)	46	25	0
Hypocalcemia (%)	9	6	0
Hyperbilirubinemia (%)	26	20	9
1-Minute Apgar score <8 (%)	40	33	23
5-Minute Apgar score <8 (%)§	14	2	5

Mean ± SEM are reported for gestational age at delivery, birth weight, and cord glucose.

*Patients with pregestational diabetes mellitus lower than nondiabetic patients ($p < 0.05$).

†Nondiabetic patients lower than patients with gestational diabetes mellitus ($p < 0.05$) and pregestational diabetes mellitus ($p < 0.05$).

‡Patients with pregestational diabetes mellitus greater than patients with gestational diabetes mellitus ($p < 0.01$) and nondiabetic patients ($p < 0.001$); patients with gestational diabetes mellitus greater than nondiabetic patients ($p < 0.02$).

§Patients with pregestational diabetes mellitus greater than patients with gestational diabetes mellitus ($p < 0.001$). All other differences among patient groups were not significant ($p > 0.05$).

diabetes mellitus and patients with pregestational diabetes mellitus vs nondiabetic patients were evaluated by the Fisher exact probability test.

These analyses showed that gestational age at delivery was significantly lower among offspring of patients with pregestational diabetes mellitus than that of offspring of nondiabetic patients ($p < 0.05$); the offspring of patients with gestational diabetes mellitus did not differ significantly from offspring of either patients with pregestational diabetes mellitus or nondiabetic patients. The incidence of preterm deliveries did not differ significantly among the three groups. Birth weight

Table IV. Partial correlations* between antepartum fuels and neonate Brazelton neonatal behavioral assessment scale responses

Dimension	*Metabolic index*	*Trimester*	
		Second	*Third*
Interactive	HbA_{1c}	($r = 0.17$, $n = 116$, $p < 0.07$)	($r = 0.14$, $n = 185$, $p < 0.06$)
	Fasting plasma glucose	($r = 0.16$, $n = 141$, $p < 0.06$)	($n = 209$, NS)
	β-hydroxybutyrate	($n = 82$, NS)	($n = 127$, NS)
Motoric	HbA_{1c}	($r = 0.20$, $n = 116$, $p < 0.04$)	($r = 0.18$, $n = 185$, $p < 0.02$)
	Fasting plasma glucose	($n = 141$, NS)	($n = 209$, NS)
	β-hydroxybutyrate	($n = 82$, NS)	($n = 127$, NS)
State control	HbA_{1c}	($n = 116$, NS)	($n = 185$, NS)
	Fasting plasma glucose	($n = 141$, NS)	($n = 209$, NS)
	β-hydroxybutyrate	($n = 82$, NS)	($n = 127$, NS)
Physiologic control	HbA_{1c}	($n = 116$, NS)	($n = 185$, NS)
	Fasting plasma glucose	($r = 0.19$, $n = 141$, $p < 0.03$)	($n = 209$, NS)
	β-hydroxybutyrate	($n = 82$, NS)	($n = 127$, NS)

*Controlling for gestational age at delivery.

was significantly greater in both diabetic groups than in the nondiabetic group (both $p < 0.05$). Whereas the incidence of macrosomia in patients with pregestational diabetes mellitus and patients with gestational diabetes mellitus was twice that in nondiabetic patients, the differences were not statistically different presumably because of the small number of nondiabetic subjects. Mean cord blood glucose levels did not differ significantly among the three groups, nor did the incidence of cord pH <7.1. The incidence of hypoglycemia was significantly increased in the group with pregestational diabetes mellitus compared with the group with gestational diabetes mellitus ($p < 0.01$) and the nondiabetic group ($p < 0.001$), and in the group with gestational diabetes mellitus compared with the nondiabetic group ($p < 0.02$). Finally, there were no significant group differences in the incidence of hypocalcemia, hyperbilirubinemia, or 1-minute Apgar scores <8. Significantly more offspring of patients with pregestational diabetes mellitus (14%) had 5-minute Apgar scores <8 than offspring of the group with gestational diabetes mellitus (2%, $p < 0.001$); whereas the incidence was 5% in the nondiabetic group (p = NS).

Brazelton neonatal behavioral assessment scale evaluations. To determine the relationship between antepartum maternal metabolism and neonate behavior, partial correlations were performed between each of the four Brazelton neonatal behavioral assessment scale dimensions (interactive, motoric, state control, and physiologic control) and the three indexes of maternal metabolism (HbA_{1C}, fasting plasma glucose, β-hydroxybutyrate) after controlling for gestational age at delivery. Partial correlation analyses were instituted because differences in the Brazelton neonatal behavioral assessment scale responses of preterm and term infants have been observed even when the preterm infants were tested at term age.[19]

Results are summarized in Table IV. Significant ($p < 0.05$) or marginally significant ($p < 0.1$) positive correlations were found between antepartum maternal glucoregulation and three of the four Brazelton neonatal behavioral assessment scale dimensions. In each instance, poorer control of maternal glucose was followed by poorer Brazelton neonatal behavioral assessment scale ratings of the neonate. Thus test scores on the interactive dimension were correlated to a marginally significant extent with second-trimester HbA_{1C} ($p < 0.07$) and fasting plasma glucose ($p < 0.06$), and third-trimester HbA_{1C} ($p < 0.06$); the motoric dimension correlated significantly with second- ($p < 0.04$) and third-trimester ($p < 0.02$) HbA_{1C}; and the physiologic control dimension correlated significantly with second-trimester fasting plasma glucose ($p < 0.03$). None of the Brazelton neonatal behavioral assessment scale dimensions correlated significantly with fasting plasma β-hydroxybutyrate.

To evaluate the effects of various perinatal factors on these metabolism-Brazelton neonatal behavioral assessment scale correlations, the partial correlation analyses were repeated with the following variables included as covariates: delivery type (i.e., cesarean vs vaginal), macrosomia, cord glucose, cord pH, neonate hypoglycemia, hyperbilirubinemia, and 1- and 5-minute Apgar scores. Results from these analyses were compared with those reported above via the procedure described by McNemar.[18] In no instance were the previously reported findings altered significantly by any perinatal factor (all $p > 0.70$).

Similarly, we evaluated the effects of ethnicity and socioeconomic status on the metabolism-Brazelton neonatal behavioral assessment scale correlations listed here by repeating the partial correlation analyses with ethnic origin (white vs minority), family income, and mother's and father's highest grade achieved as covariates. As with the perinatal factors, these analyses showed that in no instance were the previously reported findings altered significantly by ethnic or socioeconomic factors (all $p > 0.90$).

To assess for possible interactions linked to the nature of the maternal diabetes, the populations with pregestational diabetes mellitus and gestational diabetes mellitus were analyzed separately. Despite the smaller numbers and metabolic variance in these subgroups similar patterns emerged. As found in the prior analysis with the entire population, scores on the Brazelton neonatal behavioral assessment scale state control dimension did not correlate significantly with any index of antepartum maternal metabolism in the separate groups. And, significant or marginally significant correlations were obtained for the other three Brazelton neonatal behavioral assessment scale dimensions. In gravid women with pregestational diabetes mellitus, second-trimester fasting plasma glucose values correlated with neonate performance on the Brazelton neonatal behavioral assessment scale interactive ($r = 0.24, n = 64, p < 0.06$), motoric ($r = 0.22, n = 64, p < 0.09$), and physiologic control ($r = 0.26, n = 64, p < 0.04$) dimensions; and second-trimester HbA_{1C} correlated significantly with the neonate ratings on the morotic dimension ($r = 0.40, n = 52, p < 0.003$). In gravid patients with gestational diabetes mellitus, second-trimester fasting plasma glucose correlated significantly with neonate performance on the physiologic control dimension ($r = 0.37, n = 59, p < 0.004$); and third-trimester HbA_{1C} correlated significantly with neonate ratings on the motoric dimension ($r = 0.20, n = 96, p < 0.06$).

Comment

The Northwestern University Diabetes Pregnancy Center was established to conduct prospective evaluations of Freinkel's[3] proposition that maternal fuels may exert long-range effects on the progeny by modifying phenotypic gene expression in key cells during critical phases of intrauterine development (i.e., fuel-mediated teratogenesis). This manuscript reports on the observed relations between maternal fasting plasma glucose, HbA_{1C}, and β-hydroxybutyrate in the second and third trimesters and the subsequent behavioral characteristics of 209 singleton neonates. The estimates of fasting plasma glucose and β-hydroxybutyrate levels were obtained at biweekly and weekly intervals to provide indexes of glycemic control and lipid metabolism after 11 to 14 hours of dietary deprivation; the HbA_{1C} measurements were obtained monthly as an index of integrated glycemic excursions during and after meals. Estimation of HbA_{1C} had become clinically feasible at the inception of this study in 1977. Thus HbA_{1C} was chosen as a realistic alternative to random clinic measurements of postprandial plasma glucose because home glucose monitoring had not yet become a standard management procedure. The Brazelton neonatal behavioral assessment scale was chosen to gauge neurobehavior status because it has gained wide acceptance as one of the premier instruments for integrative characterization of nervous system function in the newborn period. Such characterizations may be of particular interest for metabolic correlations with antepartum events inasmuch as substantial central nervous system plasticity still obtains in the neonate and intrinsic function has not been modified by extrauterine environmental factors or interactions with care givers.

We found significant correlations between antepartum maternal glucoregulation and three of the four Brazelton neonatal behavioral assessment scale dimensions. In each case, poorer regulation of maternal glucose was followed by poorer Brazelton neonatal behavioral assessment scale ratings of the neonate. Performance on the motoric dimension correlated with second- and third-trimester HbA_{1C}; performance on the interactive dimension correlated with second- and third-trimester HbA_{1C} and second-trimester fasting plasma glucose; and performance on the physiologic control dimension correlated with second-trimester fasting plasma glucose. It should be noted that these correlations were established within a population of subjects in whom metabolic status was normal to moderately impaired. Thus even in the group with pregestational diabetes mellitus mean values for fasting plasma glucose were <105 mg/dl in the third trimester and did not exceed 231 mg/dl in any study participant. Similarly, mean β-hydroxybutyrate levels in both groups with diabetes were minimally elevated and no single measurement exceeded 0.60 mmol/L. Further, these relations persisted even when the populations with pregestational diabetes mellitus and gestational diabetes mellitus were analyzed separately. Thus insofar as most of the women with pregestational diabetes mellitus had type I diabetes, whereas women with gestational diabetes mellitus are at high risk for later development of type II diabetes, the observed correlations appear to be determined by phenotypic factors (i.e., the degree of the disturbance in the metabolism of the mother during pregnancy) rather than genotypic considerations (i.e., the nature of the mothers' diabetes). Finally, the present results cannot be ascribed to socioeconomic or ethnic variables.

Four additional factors should be considered. First, these findings are consistent with earlier reports that diabetes in the mother may affect neonate Brazelton neonatal behavioral assessment scale responses.[20, 21] However, the present study directly implicates maternal fuels for the first time and provides the additional details concerning temporal relationships, treatment schedules, and neonatal events that can only be gleaned from prospective experimental design. Second, because metabolic control across trimesters may be interrelated we cannot exclude an influence of first-trimester control in our population. Indeed, recent studies by Petersen and colleagues[22, 23] suggest that early (i.e., first trimester) intrauterine growth delay in diabetic pregnancies is associated with psychomotor deficits in the

progeny at age 4 to 5 years. Presumably such delays are derived from mothers with worse diabetic control as judged by earlier evidence that such mothers had worse HbA_{1C}.[24] Third, because glucoregulation is likely to serve as a marker for metabolic control in general, we believe it is premature to identify glucose as the principal factor in these metabolic-behavioral associations.

Finally, we emphasize that the demonstration of these relationships in diabetic patients with good-to-excellent metabolic regulation in the second and third trimesters of gestation attests to the exquisite sensitivity of developmental events to fuel-mediated influences. Given this reduced degree of metabolic variance an attenuation in the magnitude and significance of the obtained correlations was expected. However, the clincial significance of these correlations remains: (1) even relatively slight perturbations in endogenous substances during pregnancy may have deleterious effects on the fetus, and (2) maintaining tight diabetic control during pregnancy has important health benefits to the neonate. The presence of fuel-related neurobehavior deficits in neonates of diabetic mothers with poorer metabolic regulation suggests that such infants start their interactions with care givers from a modified base. Whether these neurobehavior deficits are truly teratogenic (i.e., long-lasting) or transient (i.e., subject to recovery) remains to be established from follow-up studies that are in progress.

A number of colleagues have participated in the establishment of the antepartum and peripartum management procedures at the Northwestern University Diabetes in Pregnancy Center since its inception in 1977. We are particularly grateful to senior collaborators such as Richard L. Phelps in Medicine, Drs. R. Depp, S. Dooley, R. Sabbagha, J. Sciarra, M. Socol, and R. Tamura in Obstetrics, and Drs. J. Boehm and E. Ogata in Neonatology. We are also indebted to the nursing staff of the Clinical Research Unit at Northwestern Memorial Hospital for help in the management of a number of our patients; and to Geoff Page, Ed Murillo, and past members of the Diabetes in Pregnancy Center Biometry section for help in data management and analysis.

REFERENCES

1. Freinkel N, Metzger BE. Pregnancy as a tissue culture experience: the critical implications of maternal metabolism for fetal development. In: Pregnancy metabolism, diabetes and the fetus; Ciba Foundation Symposium No. 63. Amsterdam: Excerpta Medica, 1979:3-23.
2. Freinkel N, Phelps RL, Metzger BE. Intermediary metabolism during normal pregnancy. In: Sutherland HW, Stowers JM, eds. Carbohydrate metabolism in pregnancy and the newborn. New York: Springer-Verlag 1979:1-13.
3. Freinkel N. The Banting lecture 1980: of pregnancy and progeny. Diabetes 1980;29:1023-35.
4. Freinkel N, Metzger BE, Ogata ES, et al. More concerning pregnancy as a "tissue-culture experience." In: Waldhausl WK, ed. Proceedings of the 10th Congress of the International Diabetes Foundation. Amsterdam: Excerpta Medica, 1979:731-4.
5. Metzger BE, Silverman BL, Freinkel N, Dodey SL, Ogata ES, Green OC. Amniotic fluid insulin concentration as a predictor of obesity. Arch Dis Child [In press].
6. Brazelton TB. Neonatal behavioral assessment scale. Clinics in developmental medicine, No. 50. Philadelphia: Lippincott, 1973.
7. Rizzo T, Freinkel N, Metzger BE, Hatcher R, Burns WJ, Barglow P. Fuel-mediated behavioral teratogenesis: correlations between maternal metabolism in diabetic pregnancies and Brazelton tests in the newborn [Abstract]. Diabetes 1988;37(suppl 1):86a.
8. Freinkel N. Summary and recommendations of the Second International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes 1985;34(suppl 2):123-6.
9. O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. Diabetes 1964;13:278-85.
10. Metzger BE, Phelps RL, Freinkel N, Navickas IA. Effects of gestational diabetes on diurnal profiles of plasma glucose, lipids, and individual amino acids. Diabetes Care 1980;3:402-9.
11. Li PK, Lee JT, MacGillivray MH, Schaefer PA, Siegel JH. Direct, fixed-time kinetic assays for β-hydroxybutyrate and acetoacetate with a centrifugal analyzer or a computer-backed spectrophotometer. Clin Chem 1980; 26:1713-7.
12. Trivelli LA, Ranney HM, Lai H. Hemoglobin components in patients with diabetes mellitus. N Engl J Med 1971;184:353-7.
13. Dubowitz LM, Dubowitz V, Goldberg C. Critical assessment of gestational age in the newborn infant. J Pediatr 1970;77:1-10.
14. Lubchenco LO, Hansman C, Dressler M, Boyd E. Intrauterine growth as estimated from live born birth weight data at 24 to 42 weeks of gestation. Pediatrics 1963;32: 793-800.
15. Als H, Tronick E, Lester B, Brazelton TB. Specific neonatal measures: the Brazelton neonatal behavioral assessment scale. In: Osofsky J, ed. Handbook of infant development. New York: Wiley 1979:185-215.
16. SPSS-X user's guide, 2nd ed. New York: McGraw-Hill, 1986:464-76, 477-552, 648-62.
17. Castellan NJ. On the partitioning of contingency tables. Psych Bull 1965;64:330-8.
18. McNemar Q. Psychological statistics. New York: Wiley, 1962:139-40.
19. Field T, Dempsey J, Shuman H. Developmental follow-up of pre- and postterm infants. In: Friedman S, Sigman M, eds. Preterm birth and psychological development. New York: Academic Press, 1981:299-312.
20. Yogman MW, Cole P, Als H, Lester BM. Behavior of newborns of diabetic mothers. Infant Behav Dev 1982;5:331-40.
21. DiVitto B, Goldberg S. The effects of newborn medical status on early parent-infant interaction. In: Field T, ed. Infants born at risk. New York: Spectrum 1979:311-332.
22. Petersen MB, Pedersen SA, Greisen G, Pedersen JF, Molsted-Pedersen L. Early growth delay in diabetic pregnancy: relation to psychomotor development at age 4. Br Med J 1988;296:598-600.
23. Petersen MB. Status at 4-5 years i n 90 children of insulin-dependent diabetic mothers. In: Sutherland HW, Stowers JM, Pearson DWM, eds. Carbohydrate metabolism in pregnancy and the newborn IV. London: Springer-Verlag 1989:353-361.
24. Pedersen JF, Molsted-Pedersen L, Mortensen HB. Fetal growth delay and maternal hemoglobin A_{1C} in early diabetic pregnancy. Obstet Gynecol 1984;64:351-352.



"I was devastated when I found out I had genital herpes. What a relief when my doctor recommended a treatment program including Zovirax Ointment."

Zovirax® (acyclovir) Ointment 5% is an ideal complement to any treatment program for initial herpes genitalis. As clinical studies confirm, Zovirax Ointment 5% significantly lessens the duration of pain, shortens viral shedding, *and* speeds healing.[1-3]

Whenever the comfort of a topical antiviral medication is desired, prescribe Zovirax Ointment 5% for initial herpes genitalis. It suits her needs—just when she needs it most.

In initial herpes genitalis,

ZOVIRAX®
(ACYCLOVIR)
OINTMENT 5%
Antiviral Action... Proven Results

Please see brief summary of prescribing information on next page.

References: **1.** Lacey CJN, Meaden JD, Darville JM, et al. Topical acyclovir ointment in the treatment of genital herpes simplex virus infections. *Eur J Sex Trans Dis.* 1985;2:167-170. **2.** Thin RN, Nabarro JM, Parker JD, Fiddian AP. Topical acyclovir in the treatment of initial genital herpes. *Br J Vener Dis.* 1983;59:116-119. **3.** Corey L, Benedetti JK, Critchlow CW, et al. Double-blind controlled trial of topical acyclovir in genital herpes simplex virus infections. Acyclovir symposium. *Am J Med.* 1982;73:326-334.

ZOVIRAX®
(ACYCLOVIR)
OINTMENT 5%

Before prescribing, please consult full package insert, a summary of which follows:

INDICATIONS AND USAGE: Zovirax (Acyclovir) Ointment 5% is indicated in the management of initial herpes genitalis and in limited nonlife-threatening mucocutaneous Herpes simplex virus infections in immunocompromised patients. In clinical trials of initial herpes genitalis, Zovirax Ointment 5% has shown a decrease in healing time and in some cases a decrease in duration of viral shedding and duration of pain. In studies in immunocompromised patients with mainly herpes labialis, there was a decrease in duration of viral shedding and a slight decrease in duration of pain.

By contrast, in studies of recurrent herpes genitalis and of herpes labialis in nonimmunocompromised patients, there was no evidence of clinical benefit; there was some decrease in duration of viral shedding.

Diagnosis: Whereas cutaneous lesions associated with Herpes simplex infections are often characteristic, the finding of multinucleated giant cells in smears prepared from lesion exudate or scrapings may assist in the diagnosis.[1] Positive cultures for Herpes simplex virus offer a reliable means for confirmation of the diagnosis. In genital herpes, appropriate examinations should be performed to rule out other sexually transmitted diseases.

CONTRAINDICATIONS: Zovirax Ointment 5% is contraindicated for patients who develop hypersensitivity or chemical intolerance to the components of the formulation.

WARNINGS: Zovirax Ointment 5% is intended for cutaneous use only and should not be used in the eye.

PRECAUTIONS:

General: The recommended dosage, frequency of applications, and length of treatment should not be exceeded (see DOSAGE AND ADMINISTRATION). There exist no data which demonstrate that the use of Zovirax Ointment 5% will either prevent transmission of infection to other persons or prevent recurrent infections when applied in the absence of signs and symptoms. Zovirax Ointment 5% should not be used for the prevention of recurrent HSV infections. Although clinically significant viral resistance associated with the use of Zovirax Ointment 5% has not been observed, this possibility exists.

Drug Interactions: Clinical experience has identified no interactions resulting from topical or systemic administration of other drugs concomitantly with Zovirax Ointment 5%.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Acyclovir was tested in lifetime bioassays in rats and mice at single daily doses of 50, 150 and 450 mg/kg/day given by gavage. These studies showed no statistically significant difference in the incidence of benign and malignant tumors produced in drug-treated as compared to control animals, nor did acyclovir induce the occurrence of tumors earlier in drug-treated animals as compared to controls. In 2 *in vitro* cell transformation assays, used to provide preliminary assessment of potential oncogenicity in advance of these more definitive lifetime bioassays in rodents, conflicting results were obtained. Acyclovir was positive at the highest dose used in one system and the resulting morphologically transformed cells formed tumors when inoculated into immunosuppressed, syngeneic, weanling mice. Acyclovir was negative in another transformation system.

No chromosome damage was observed at maximum tolerated parenteral doses of 100 mg/kg acyclovir in rats or Chinese hamsters; higher doses of 500 and 1000 mg/kg were clastogenic in Chinese hamsters. In addition, no activity was found in a dominant lethal study in mice. In 9 of 11 microbial and mammalian cell assays, no evidence of mutagenicity was observed. In 2 mammalian cell assays (human lymphocytes and L5178Y mouse lymphoma cells *in vitro*), positive response for mutagenicity and chromosomal damage occurred, but only at concentrations at least 1000 times the plasma levels achieved in man following topical application.

Acyclovir does not impair fertility or reproduction in mice at oral doses up to 450 mg/kg/day or in rats at subcutaneous doses up to 25 mg/kg/day. In rabbits given a high dose of acyclovir (50 mg/kg/day, s.c.), there was a statistically significant decrease in implantation efficiency.

Pregnancy: *Teratogenic Effects*. Pregnancy Category C. Acyclovir was not teratogenic in the mouse (450 mg/kg/day, p.o.), rabbit (50 mg/kg/day, s.c. and i.v.) or in standard tests in the rat (50 mg/kg/day, s.c.). In a non-standard test in rats, fetal abnormalities, such as head and tail anomalies, were observed following subcutaneous administration of acyclovir at very high doses associated with toxicity to the maternal rat. The clinical relevance of these findings is uncertain.[2] There are no adequate and well-controlled studies in pregnant women. Acyclovir should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Zovirax is administered to a nursing woman.

ADVERSE REACTIONS: Because ulcerated genital lesions are characteristically tender and sensitive to any contact or manipulation, patients may experience discomfort upon application of ointment. In the controlled clinical trials, mild pain (including transient burning and stinging) was reported by 103 (28.3%) of 364 patients treated with acyclovir and by 115 (31.1%) of 370 patients treated with placebo; treatment was discontinued in 2 of these patients. Other local reactions among acyclovir-treated patients included pruritus in 15 (4.1%), rash in 1 (0.3%) and vulvitis in 1 (0.3%). Among the placebo-treated patients, pruritus was reported by 17 (4.6%) and rash by 1 (0.3%).

In all studies, there was no significant difference between the drug and placebo group in the rate or type of reported adverse reactions nor were there any differences in abnormal clinical laboratory findings.

OVERDOSAGE: Overdosage by topical application of Zovirax Ointment 5% is unlikely because of limited transcutaneous absorption (see Clinical Pharmacology).

DOSAGE AND ADMINISTRATION: Apply sufficient quantity to adequately cover all lesions every 3 hours 6 times per day for 7 days. The dose size per application will vary depending upon the total lesion area but should approximate a one-half inch ribbon of ointment per 4 square inches of surface area. A finger cot or rubber glove should be used when applying Zovirax to prevent autoinoculation of other body sites and transmission of infection to other persons. **Therapy should be initiated as early as possible following onset of signs and symptoms.**

HOW SUPPLIED: Zovirax Ointment 5% is supplied in 15 g tubes (NDC 0081-0993-94) and 3 g tubes (NDC 0081-0993-41). Each gram contains 50 mg acyclovir in a polyethylene glycol base. Store at 15°-25°C (59°-77°F) in a dry place.

REFERENCES

1. Naib ZM et al. *Cancer Res* 33:1452-1463, 1973.
2. Stahlmann R, Klug S, Lewandowski C, et al. *Infection:* 15(4): 261-262, 1987.

U.S. Patent No. 4199574

IMPROVING LIVES THROUGH ANTIVIRAL RESEARCH

Burroughs Wellcome Co.,
Research Triangle Park,
North Carolina 27709

Copr. © 1989 Burroughs Wellcome Co. All rights reserved.

ZO-1029

Mosby Year Book

MEDICAL JOURNALS

Dedicated to Publishing Excellence

SAMPLE COPIES OF JOURNALS ARE AVAILABLE

* AMERICAN HEART JOURNAL
Monthly. Institutions: $125.00; Individuals: $65.00.

* AMERICAN JOURNAL of INFECTION CONTROL
Bimonthly. Institutions: $82.00; Individuals: $23.75.

* AMERICAN JOURNAL of OBSTETRICS and GYNECOLOGY
Monthly. Institutions: $130.00; Individuals: $71.50.

* CLINICAL PHARMACOLOGY & THERAPEUTICS
Monthly. Institutions: $141.00; Individuals: $77.00.

* HEART & LUNG: THE JOURNAL of CRITICAL CARE
Bimonthly. Institutions: $85.00; Individuals: $24.50.

* HEPATOLOGY
Monthly. Institutions: $205.00; Individuals: $150.00.

* THE JOURNAL of ALLERGY and CLINICAL IMMUNOLOGY
Monthly. Institutions: $116.50; Individuals: $53.50.

* JOURNAL of the AMERICAN ACADEMY of DERMATOLOGY
Monthly. Institutions: $130.00; Individuals: $69.00.

JOURNAL of the AMERICAN ACADEMY of PHYSICIAN ASSISTANTS
Eight times a year. Institutions: $50.00; Individuals: $32.00.

* JOURNAL of the AMERICAN SOCIETY of ECHOCARDIOGRAPHY
Bimonthly. Institutions: $56.00; Individuals: $40.00.

* JOURNAL of BURN CARE & REHABILITATION
Bimonthly. Institutions: $46.25; Individuals: $30.00.

* THE JOURNAL of HAND SURGERY (American Volume)
Bimonthly. Institutions: $108.00; Individuals: $46.50.

* THE JOURNAL of HEART TRANSPLANTATION
Bimonthly. Institutions: $72.50; Individuals: $46.00.

* THE JOURNAL of LABORATORY and CLINICAL MEDICINE
Monthly. Institutions: $138.00; Individuals: $73.50.

* THE JOURNAL of PEDIATRICS
Monthly. Institutions: $120.50; Individuals: $59.00.

* THE JOURNAL of THORACIC and CARDIOVASCULAR SURGERY
Monthly. Institutions: $141.00; Individuals: $81.50.

* JOURNAL of VASCULAR SURGERY
Monthly. Institutions: $124.00; Individuals: $67.00.

* OTOLARYNGOLOGY—HEAD and NECK SURGERY
Monthly. Institutions: $143.00; Individuals: $77.00.

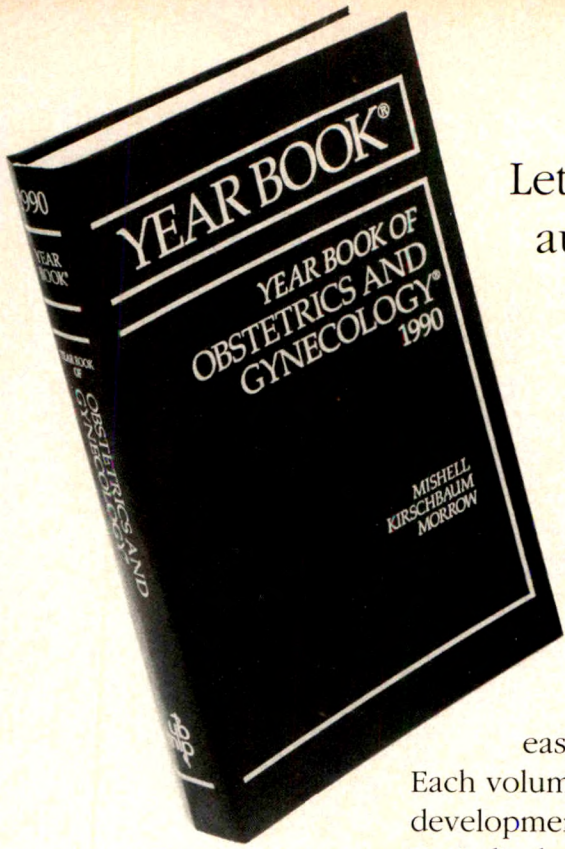
* SURGERY
Monthly. Institutions: $123.00; Individuals: $60.00.

* Included in *Index Medicus* J9850FZC

Subscription rates quoted are for USA only. International rates (surface and airmail delivery) available upon request. All subscriptions are for one-year terms. Subscriptions may begin at any time. To order your subscription and for more information, contact:

MOSBY-YEAR BOOK, INC.
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, Missouri 63146 U.S.A.

Call:
(314) 872-8370, ext. 7351
In the U.S., call toll-free:
1-800-325-4177, ext. 7351



Let our eminent medical authorities guide you through major new clinical advances

Introducing the

Year Book of Obstetrics & Gynecology®

This time-saving guide book makes it easy for you to keep current in your field. Each volume represents the year's most significant developments as reported in more than 800 journals and periodicals published worldwide. These selections are condensed to roughly a page in length, so you get all essential facts at a glance.

More importantly, the distinguished physician editors provide thoughtful, probing commentaries that explore the significance of each article while evaluating its practical implications for day-to-day practice.

Editors: Daniel R. Mishell, Jr., M.D.; Thomas H. Kirschbaum, M.D.; and C. Paul Morrow, M.D. **Contributing Editor**: William H. Hindle, M.D.

These books are published annually. For the convenience of its customers, Year Book enters each purchaser as a subscriber to future volumes and sends annual announcements of each volume approximately two months before publication. The new volume will be shipped upon publication unless you complete and return the cancellation notice attached to the announcement and it is received by Year Book within the time indicated (approximately 20 days after your receipt of the announcement). You may cancel your subscription at any time. The new volume may be examined on approval for 30 days and returned for full credit. Year Book will remove your name as a subscriber. Return postage is guaranteed by Year Book to the Postal Service.

To Subscribe:

Simply fill out this coupon and send it to us at the address shown.

Or,

for faster service, call our TOLL-FREE number:

1-800-426-4545

Prepaid orders are shipped postage free; add $3.50 per order to cover handling. Other orders will be billed a shipping and handling charge. IL, MA, TN residents will be billed appropriate sales tax. Prices are quoted in U.S. dollars. Canadian orders payable in U.S. funds. All prices subject to change without notice.

[X] ***YES!***

I know it's vital that I stay informed. Please rush me the most recent issue of the *Year Book of Obstetrics & Gynecology* (5-90) for $54.95.

❒ Payment Enclosed ❒ Please bill me ❒ Charge my Amex/Visa/MasterCard (circle one)

Signature

Account Number/Expiration Date

Name

Address

City/State/Zip

Telephone XX9

Mail to:

Mosby-Year Book Inc.

Order Processing, 11830 Westline Industrial Dr.

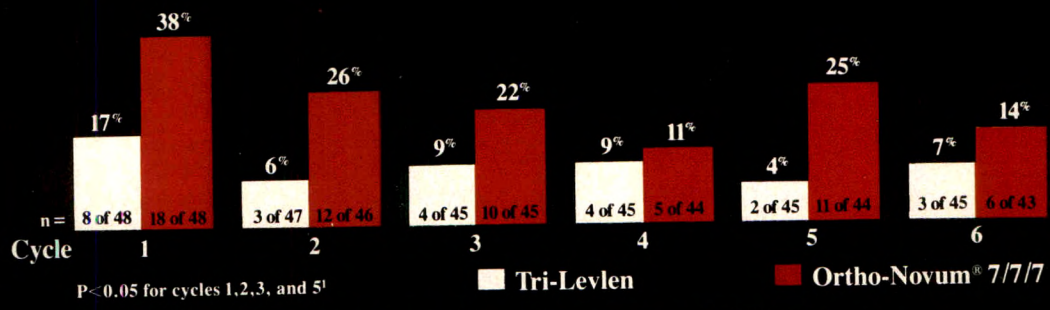
St. Louis, MO 63146

A red spot means a lot to a woman.

A red spot may mean breakthrough bleeding.

Tri-Levlen® means a low incidence of breakthrough bleeding/spotting*†

Multicenter study demonstrates significantly less breakthrough bleeding/spotting through the first six cycles[1]



P<0.05 for cycles 1,2,3, and 5[1]

n = number of women with a bleeding event occurring during a cycle.

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen

may mean a lot to a woman

* Incidence defined as percentage of women with a bleeding event occurring at least once during a pill cycle. Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

† Defined as intermenstrual bleeding.

Ortho-Novum® is a registered trademark of Ortho Pharmaceutical Corp.

BERLEX Dedicated to innovation in OB/GYN medicine

TRI-LEVLEN® 28 Levonorgestrel and ethinyl estradiol
TRI-LEVLEN® 21 tablets–Triphasic regimen

BRIEF SUMMARY
Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5) 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted.: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

Reference: 1. Data on file, Berlex Laboratories, Inc.

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets–
Triphasic regimen

BERLEX
Dedicated to innovation in OB/GYN medicine

BERLEX Laboratories, Inc., Wayne, New Jersey 07470

© 1990, Berlex Laboratories, Inc. All rights reserved.

430-200

Antenatal microbiologic and maternal risk factors associated with prematurity

James A. McGregor, MDCM,[a] Janice I. French, CNM, MS,[a] Ruth Richter, CNM, MSN,[a] Amalia Franco-Buff, MS,[a] Anne Johnson, MS,[a] Sharon Hillier, PhD,[b] Franklyn N. Judson, MD,[c, d] and James K. Todd, MD[e]

Denver, Colorado, and Seattle, Washington

In a prospective study of 202 women (gestational age 24 ± 4 weeks), we evaluated possible influences of lower genital tract infection or bacterial conditions on obstetric outcomes, including preterm labor, preterm premature rupture of membranes, and preterm birth. The presence of bacterial vaginosis (18.7%) was associated with an increased risk of preterm labor (relative risk, 2.6; 95% confidence interval, 1.08 to 6.46). For women with bacterial vaginosis who also had *Mobiluncus species* morphotypes identified on Gram stain, the relative risk of preterm labor was 3.8 (95% confidence interval, 1.32 to 11.5). Presence of vaginal *Mycoplasma hominis* (10.8% of patients) was associated with both preterm labor (relative risk, 1.8; 95% confidence interval, 0.77 to 4.4) and preterm birth (relative risk, 5.1; 95% confidence interval, 1.45 to 17.9). Recovery of *Staphylococcus aureus* (3.0%) was associated with preterm labor (relative risk, 3.1; 95% confidence interval 1.12 to 8.7). Identification of two or more bacterial-linked abnormalities was also associated with preterm labor (relative risk, 3.3; 95% confidence interval, 1.44 to 7.58). An increased level of vaginal wash protease (≥10 trypsin units) (16%) was associated with preterm labor and was noted in 50% of women with preterm premature rupture of membranes. A history of prior preterm birth was the single best historical predictor of both preterm labor (relative risk, 3.6; 95% confidence interval, 1.92 to 6.83) and preterm birth (relative risk, 6.7; 95% confidence interval, 2.2 to 20.4). History of three or more abortions, antenatal urinary tract infection, and occurrence of medical complications during pregnancy also correlated with increased risk of preterm labor. These findings affirm and refine associations of various maternal reproductive tract infections with preterm labor, premature rupture of membranes, and birth, allowing for controlled treatment trials aimed at prevention of preterm birth. (AM J OBSTET GYNECOL 1990;163:1465-73.)

Key words: Prematurity, preterm birth, bacteria, infection, risks, pathogenesis

Preterm labor with subsequent delivery of premature newborns remains the largest cause of perinatal morbidity and mortality worldwide.[1] Much past work has focused on identification of maternal demographic, behavioral, obstetric, and medical factors associated with increased risk of preterm labor and birth. Disappointingly, interventions on the basis of these risk factors have yielded only inconsistent results.[2-4] To be optimally effective in the reduction of risks of preterm birth, intervention strategies should be based on understanding of the pathophysiologic processes involved and focus on safe treatment of specifically implicated etiologic factors.

Much epidemiologic, clinical, and laboratory information indicates that reproductive tract infection and the inflammatory responses engendered are linked to some instances of preterm labor, preterm rupture of membranes (PROM), and preterm birth.[5-15] Studies have shown that women who have idiopathic preterm labor frequently have one or more of these findings: (1) increased evidence of histologic chorioamnionitis[6, 9]; (2) increased recovery of certain microorganisms from the fetal membranes or placenta[7, 8]; (3) increased recovery of microorganisms, bacterial endotoxin, or inflammatory mediators from amniotic fluid.[8, 10]

Attempts to identify and correlate the presence of specific lower genital tract microflora or bacterial conditions, such as bacterial vaginosis, with preterm birth or PROM have yielded inconclusive findings. A variety of lower reproductive tract microorganisms, including *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis,* group B streptococcus, *Mycoplasma hominis,*

From the Department of Obstetrics and Gynecology, University of Colorado Health Sciences Center,[a] Department of Obstetrics and Gynecology, University of Washington,[b] Denver Department of Health and Hospitals,[c] Department of Medicine and Preventive Medicine, University of Colorado Health Sciences Center,[d] and Department of Pediatrics, University of Colorado Health Sciences Center.[e]
Received for publication April 24, 1990; revised July 2, 1990; accepted July 27, 1990.
Reprint requests: James A. McGregor, MDCM, University of Colorado Health Sciences Center, Box B198, 4200 E. 9th Ave., Denver, CO 80262.
6/1/24146

Ureaplasma urealyticum, and various anaerobic flora as well as bacterial vaginosis, have been linked to preterm birth in case-controlled studies.[7-9, 11-15] Such studies have been difficult to interpret because: (1) different microorganisms or conditions are sought in various investigations; (2) techniques for screening and identification of microorganisms differ; (3) the prevalence of microorganisms varies in different populations; or (4) associations with multiple microorganisms are not sought. In addition to lower genital tract microflora, the presence of specific cumulative microbial-host interactions that may lead to increased local tissue levels of possible virulence factors, such as phospholipases or proteases, may also play roles in the pathogenesis of preterm labor or PROM.

Therefore we undertook a prospective study to evaluate possible influences of lower reproductive tract microorganisms or bacterial conditions implicated in the occurrence of preterm labor or PROM. Wash samples for measurement of nonspecific vaginal fluid proteases were also evaluated. To increase the applicability of possible results, we used only widely available and relatively inexpensive microbiologic techniques. Detailed aspects of demographic, obstetric, and medical information were collected and correlated with microbiologic findings and reproductive outcomes.

Patients and methods

Patient population. Between January 1985 and April 1986, 202 pregnant women receiving care at two publicly supported prenatal clinics in Denver were enrolled and prospectively followed up with their infants through 6 weeks post partum. Patients were enrolled at approximately 24 weeks' gestation. Women with known multiple gestation, cervical cerclage, placenta previa, history of vaginal bleeding during pregnancy, or history of preterm labor occurring in this pregnancy were excluded. Women were also excluded if within the previous 2 weeks they have received vaginal or systemic antimicrobial treatment, if they reported history of douching within 24 hours of examination, or if they were unwilling or unable to sign informed consent. Six patients were excluded from analysis because they were lost to follow-up for the remainder of the pregnancy or for delivery information. Placenta previa was diagnosed in three women subsequent to enrollment; these women remained in the study. A total of 196 pregnant women and newborns were evaluated. This study was reviewed and approved by the Human Subjects Committee of the University of Colorado Health Sciences Center.

Methods. On admission to the study, patients were interviewed to obtain detailed demographic, obstetric, and medical histories, and to complete the Creasy risk of preterm delivery scale.[2] A sterile speculum examination was performed; characteristics of vaginal discharge (consistency, amount, color, odor) were recorded on standard forms. Sterile cotton swabs that had been previously demonstrated nontoxic for chlamydial and *Mycoplasma* cultures were used to collect specimens. Samples obtained were endocervical specimens for culture of *N. gonorrhoeae, C. trachomatis,* and Microtrak (Syva Corp., Palo Alto, Calif.) chlamydia antigen, and midvaginal cotton swab cultures for *M. hominis, U. urealyticum,* group A streptococcus, group B streptococcus, *Staphylococcus aureus, Gardnerella vaginalis,* and yeast species. Because of new information during the course of the study, a vaginal smear for Gram stain evaluation of bacterial vaginosis and *Mobiluncus species* morphotypes and vaginal fluid pH were added to the test procedures. Vaginal fluid pH was assessed with Nitrazine (E. R. Squibb and Sons, Inc., Princeton, N. J.) paper and recorded as >4.5 or ≤ 4.5. One milliliter of saline solution was inoculated with a cotton swab saturated with vaginal fluid and was examined microscopically for *Trichomonas vaginalis* and yeast, and a standardized, semiquantitative assessment of vaginal leukocytes and bacteria was carried out. A drop of vaginal fluid was combined with a drop of 10% potassium hydroxide to test for release of amine odor and for further microscopic examination for yeast pseudohyphae. Finally, the posterior vaginal fornix was irrigated with 5 ml of sterile Dulbecco's phosphate buffered saline solution and, after standardized mixing, aspirated into a sterile syringe. These vaginal wash specimens were frozen immediately at $-20°$ C and then frozen at $-70°$ C within 4 hours of collection.

Culture specimens were immediately inoculated on standard, easily available media in the examination room. Endocervical cotton swabs were inoculated onto chocolate agar and modified Thayer-Martin medium split plates and held in a carbon dioxide environment for the recovery of *N. gonorrhoeae.* A separate endocervical swab was inoculated into sucrose-phosphate glutamate transport media for recovery of *C. trachomatis.* Additional cervicovaginal swabs were inoculated onto 5% sheep's blood agar (tryptic soy base, Remel, Denver, Colo.) for identification of group A streptococcus, group B streptococcus, *S. aureus, G. vaginalis,* and yeast, and into a standard mycoplasma medium for recovery of *M. hominis,* and *U. urealyticum.* Specimens were held at 4° C or at room temperature as appropriate and were transported to the laboratory within 4 hours of collection. Evaluation for *T. vaginalis,* yeast pseudohyphae, and amine odor were performed in the clinic setting. The remainder of the saline solution preparation was held at 4° C and evaluated microscopically for leukocytes and bacteria semiquantitatively in the laboratory. In addition, the saline solution wet preparation was semiquantitatively tested for nonspecific protease activity using the Azocoll substrate.[16] Cultures for *C. trachomatis, M. hominis,* and *U. urealyti-*

cum specimens were frozen at −70° C and processed in batches.

Standard techniques were used for culture of *C. trachomatis.*[17] After 48 hours incubation at 35° C, inoculated cycloheximide-treated McCoy cell monolayers were treated with fluorescence-conjugated antichlamydia monoclonal antibody (Microtrak, Syva Corp.) and examined microscopically for the presence of stained inclusions. Negative cultures were blind passaged once and reprocessed. Chlamydia antigen preparations were processed as recommended by the manufacturer. *M. hominis* and *U. urealyticum* were cultured and identified with the Mycotrim GU triphasic culture system (HANA Biologics, Inc., Alameda, Calif.). *G. vaginalis* were recovered by reincubating the chocolate–Thayer-Martin agar plate anaerobically for 3 days after examination for *N. gonorrhoeae.* Microorganisms were identified with standard techniques.[17]

Gram stain evaluation for bacterial vaginosis and *Mobiluncus species* morphotypes was performed using the method and criteria of Spiegel et al.[18] Gram stains were evaluated by one of the investigators (S. H.) who remained blinded to patient symptoms, findings, and outcomes.

After enrollment, patients continued routine prenatal care. None of the patients was seen in special clinics for preterm birth prevention and microbiologic evaluations were not repeated as part of the study protocol. Microbiologic findings were not known to the patients' health care providers. A single patient from whom *N. gonorrhoeae* was recovered was treated with a standard oral therapy specific for this infection. After birth and completion of the 6 weeks follow-up, maternal and newborn medical records were reviewed to obtain information on pregnancy and newborn course. If records were unavailable, the data were obtained by telephone calls and written correspondence to private physicians and patients.

Patients were considered to have preterm labor if the medical records reported hospital evaluation for regular uterine contractions before 37 weeks' gestation for which patients received treatment (e.g., parenteral or oral tocolytic treatment or intravenous hydration and bed rest, or contractions that resulted in preterm birth). Preterm birth was defined as birth before completion of 37 weeks' gestation. Gestational age was determined by overall "best" obstetric criteria that included history of last menstrual period, clinical dating criteria (uterine size at first examination, gestational age at detection of fetal heart rate by fetoscope, growth of fundal height), and ultrasonographic measurements, as well as newborn examination.[19] Determination of gestational age was performed in a blinded fashion before correlation with any other study determination. Preterm premature rupture of membranes was defined as rupture of fetal membranes before 37 weeks' gestation and >1 hour before the onset of labor.

Data were analyzed with two-tailed χ^2 or Fisher's exact tests for nonparametric data and the Student's *t* test for normally distributed parametric data. Relative risks (RR) and 95% confidence intervals (CI) were also calculated with SPSS (SPSS Inc., Chicago). Variables associated with preterm birth with univariate analysis or those that had been frequently associated with preterm birth in other studies were included in a logistic regression model.

Results

The majority (143) of participants were of northern European ancestry (70.8%), 21 (10.4%) were black, 32 (15.8%) were Hispanic, and 6 (3%) were of Native American or Asian descent. The mean maternal age was 23.5 ± 4.7 years (Table I). A total of 42% were nulliparous and 67% were married; all were of low socioeconomic status attending publicly supported clinics. The mean gestational age at enrollment was 24.2 ± 4.7 (SD) weeks. Preterm labor occurred among 33 (16.8%) of the patients. Twenty (60.6%) of these patients received parenteral tocolytic therapy for preterm labor. The mean gestational age at diagnosis of preterm labor was 29.2 ± 5.3 weeks. Nine patients (4.5%) were delivered of preterm infants (≤36 weeks) at a mean gestational age of 35.4 ± 0.7 weeks. Preterm PROM occurred among four (2.1%) study patients. One of the three women with placenta previa detected after study enrollment was seen for preterm labor and she was treated with intravenous hydration and bed rest only; all three women with placenta previa were delivered of infants at term. Preterm labor or delivery did not occur in any of the six (3.1%) patients in whom pregnancy-induced hypertension developed, the two patients who had abruptio placentae, or the two who were treated for clinical chorioamnionitis. There were no perinatal or neonatal deaths. Three infants were born with minor congenital anomalies. Seven infants (3.6%) were small for gestational age and 30 (16.8%) were large for gestational age.

The women in whom preterm labor or preterm rupture of membranes developed or who were delivered of preterm infants and those who did not suffer these complications did not differ in any demographic or behavioral factor except for reported use of recreational "street drugs" (Table I). These women were significantly less likely to suffer preterm labor or delivery. Marijuana was the most common recreational drug; one patient reported additional use of both methamphetamine and cocaine.

Several medical, obstetric, historical, and antenatal factors were associated with the occurrence of preterm labor or birth (Table II). History of prior preterm birth

Table I. Comparison of maternal demographic and behavioral characteristics for women who experienced preterm labor and preterm birth and those who did not

	Preterm labor (N = 33)		*Term labor (N = 163)*		*Preterm delivery (N = 9)*		*Term delivery (n = 187)*	
	n	%	*n*	%	*n*	%	*n*	%
Maternal age (yr)								
15-19	7	21.2	40	24.5	1	11	45	24.1
20-29	20	60.6	109	66.9	6	66.7	124	66.3
30-38	6	18.2	14	8.6	2	22.2	18	9.6
Not married	12	36.4	51	31.3	3	37.5	60	31.7
Nonwhite race	8	24.2	49	30.1	3	37.5	54	28.5
Nulliparous	10	30.3	73	44.8	2	25	81	42.9
Parity ≥ 1	23	69.7	90	55.2	6	75	108	57.1
Cigarette smoking	8	24.2	44	27	2	25	50	26.5
Street drugs use	0		24	14.7*	0		24	12.8

*$p < 0.01$.

Table II. Maternal historical and antepartum risk factors associated with preterm labor and delivery

	Labor							*Delivery*						
	Preterm		*Term*					*Preterm*		*Term*				
	n	%	*n*	%	*p Value*	*Relative risk*	*95% Confidence interval*	*n*	%	*n*	%	*p Value*	*Relative risk*	*95% Confidence interval*
Maternal medical problems	10	33.3	20	66.7	0.01	2.4	1.25-4.64	3	37.5	27	14.3	0.105	3.34	0.889-12.55
>3 Abortions	6	18.2	8	4.9	0.007	2.9	1.34-6.25	1	11.1	14	7.4	0.52	1.5	0.20-11.4
Prior preterm birth	10	30.3	11	6.8	0.001	3.6	1.92-6.83	4	44.4	17	9.04	0.01	6.7	2.2-20.4
Creasy risk score >10	21	63.6	64	39.3	0.01	2.28	1.22-4.29	6	66.7	80	42.6	0.2	2.6	0.7-9.5
Antepartum treatment for urinary tract infection	8	24.2	19	11.8	0.06	1.98	0.97-4.04	2	22.2	25	13.4	0.36	1.8	0.39-8.1
Antepartum treatment for nonyeast vaginitis	10	30.3	27	16.8	0.07	1.84	0.95-3.6	0		37	19.9	0.21	—	—

was the single best historical predictor of both preterm labor (RR, 3.6; 95% CI, 1.92 to 6.83) and preterm birth (RR, 6.7; 95% CI, 2.2 to 20.4). A Creasy risk index score of ≥10 was significantly associated with development of preterm labor ($p < 0.05$; RR, 2.28; 95% CI, 1.22 to 4.29). The RR of preterm labor for women with any medical problem was 2.4 (95% CI, 1.25 to 4.64). Medical problems experienced by the women in this sample included asthma requiring medication, prior pyelonephritis or frequent urinary tract infections, chronic hypertension, mitral valve stenosis, class III heart disease, and gestational diabetes. Women with three or more abortions (spontaneous or elective) had a RR of preterm labor of 2.9 (95% CI, 1.34 to 6.25). History of prior preterm birth, increased Creasy risk index, or medical problems was not associated with any microbiologic finding.

Recovery rates for microbiologic samples are shown in Table III along with microorganisms associated with preterm labor and delivery. As noted, the patient from whom *N. gonorrhoeae* was recovered received single-dose antibiotic treatment. *M. hominis* was recovered in 10.8% of patients, and was significantly associated with premature delivery ($p < 0.05$; RR, 5.1; 95% CI, 1.45 to 17.9). *U. urealyticum* was recovered from 64% of study women and was not significantly associated with preterm labor or delivery. Two (15.4%) of the *C. trachomatis* culture-positive women were delivered of preterm infants compared with seven (3.8%) of the chlamydia culture-negative women ($p = 0.09$; RR, 4.0; 95% CI, 0.97 to 16.7). Semiquantitative examination for vaginal white blood cells or bacteria did not correlate with obstetric outcomes.

Gram stain evaluation of vaginal fluid detected bacterial vaginosis among 18.7% (26) of the evaluated patients ($N = 139$). *Mobiluncus species* morphotypes were identified among eight (30.8%) of these women with bacterial vaginosis or 5.8% of those examined. Bacterial

Table III. Association between recovered microorganisms and preterm labor and preterm birth

	No. positive/no. sampled (%)	Labor							Delivery						
		Preterm		Term		p Value	Relative risk	95% Confidence interval	Preterm		Term		p Value	Relative risk	95% Confidence interval
		n	%	n	%				n	%	n	%			
Bacterial vaginosis	26/139 (18.7)	6	35.3	17	14.5	0.03	2.6	1.08-6.46	1	25	23	17.6	0.7	1.5	0.167-14.2
Mobiluncus species	8/139 (5.8)	3	17.7	4	3.4	0.04	3.8	1.32-11.5	0	—	7	5.3	0.8	—	—
Mycoplasma hominis	19/176 (10.8)	5	17.9	13	9.2	0.17	1.8	0.77-4.4	3	37.5	15	9.2	0.04	5.1	1.45-17.94
Ureaplasma urealyticum	112/176 (64)	21	75	87	61.3	0.17	1.7	0.8-3.7	7	87.5	102	62.6	0.26	3.98	0.6-26.54
Chlamydia trachomatis	13/200 (6.5)	3	9.1	10	6.2	0.46	1.4	0.48-4.1	2	22.2	11.1	5.9	0.09	4	0.97-16.66
Staphylococcus aureus	6/199 (3.0)	3	9.1	3	1.9	0.06	3.1	1.12-8.7	1	11.1	5	2.7	0.25	3.92	0.59-25.81
Streptococcus, group B	7/202 (3.5)	1	3.03	6	3.7	1	0.8	0.14-6.2	0		7	3.7	1	—	—
Gardnerella vaginalis	41/193 (21.2)	9	27.3	32	20.0	0.35	1.4	0.69-2.79	3	33.3	39	21.08	0.4	1.81	0.48-6.89
Yeast	83/196 (42.4)	13	29.4	70	42.9	0.7	0.9	0.47-1.7	3	33.3	81	43.1	0.7	0.67	0.18-2.59

vaginosis was detected among 35% of these Gram stain–evaluated women who had preterm labor as compared with 14.5% of the women who did not have preterm labor. The RR of preterm labor for women with bacterial vaginosis was 2.6 (95% CI, 1.08 to 6.46). For those women with bacterial vaginosis who also had *Mobiluncus species* morphotypes present, the RR for preterm labor was 3.8 (95% CI, 1.32 to 11.5).

S. aureus was identified among six (3.0%) patients. Preterm labor occurred in three of these women ($p = 0.06$; RR, 3.1; 95% CI, 1.12 to 8.7). Group B streptococcus was recovered from seven (3.5%) of the women and was not associated with preterm labor or preterm delivery. Three of these women had group B streptococcus identified independently by their care providers, and received treatment.

Approximately 29% (28.6%) of study participants had two or more of the microorganisms or conditions sought in this study. Identification of two or more positive cervicovaginal isolates or conditions was associated with increased risk of preterm labor (RR, 3.3; CI, 1.44 to 7.58). As would be expected, bacterial vaginosis was significantly associated with detection of *Mobiluncus species* morphotypes, *G. vaginalis,* and *M. hominis* ($p < 0.05$). Although frequently recovered, *U. urealyticum* was not statistically associated with other microbiologic findings. Recovery of group B streptococcus and *S. aureus* were also not statistically associated with other microbiologic findings; however, the numbers are small. Neither *T. vaginalis* nor group A streptococcal infection was identified with these methods.

Vaginal wash nonspecific protease levels ≥10 trypsin units/ml were detected in 32/196 (16%) of the women. Increased vaginal protease activity was noted among two of four (50%) women who had preterm PROM. Overall, findings of ≥10 trypsin units/ml were not associated with increased risk of preterm birth. Among the 47 women tested, Nitrazine paper determination of vaginal pH >4.5 or ≤4.5 was not associated with abnormal outcome.

Recovery of *M. hominis* was more common among nonwhite participants (18.2%) than among white participants (7.4%) ($p < 0.05$). Other associations between ethnicity and microorganisms recovered were not identified. Detection of bacterial vaginosis, *Mobiluncus species, M. hominis,* and *C. trachomatis* occurred more often among unmarried women ($p < 0.1$).

Fully 48% of the study patients received some form of antimicrobial therapy during their antenatal care. Twenty-eight (14.2%) were treated for a single urinary tract infection. Women who had one or two urinary tract infections had a higher incidence of preterm labor ($p < 0.06$; RR, 1.98; 95% CI, 0.97 to 4.04). These women were asymptomatic and had negative test of cure urine cultures after treatment. Women who had more than two lower urinary tract infections ($n = 4$) or pyelonephritis ($n = 3$) received oral antibiotic treatment daily until delivery and did not have preterm labor or birth. Review of antenatal records revealed that 36 (18.3%) study patients were treated for yeast vaginitis; and 18.8% received treatment of vaginitis other than yeast. Two (1%) patients were treated for *N. gonorrhoeae* (a further patient received single-dose treatment after enrollment), 5.1% for *T. vaginalis,* 9.6% for *G. vaginalis,* 4.6% for cervicitis, 0.5% for *C. trachomatis,* and 2% for group B streptococcus. Preterm labor occurred more often among women who received treatment of *Gardnerella vaginalis*–associated vaginitis ($p < 0.05$) from their clinical care providers. Nevertheless, fully 82% (27/33) of the women who had pre-

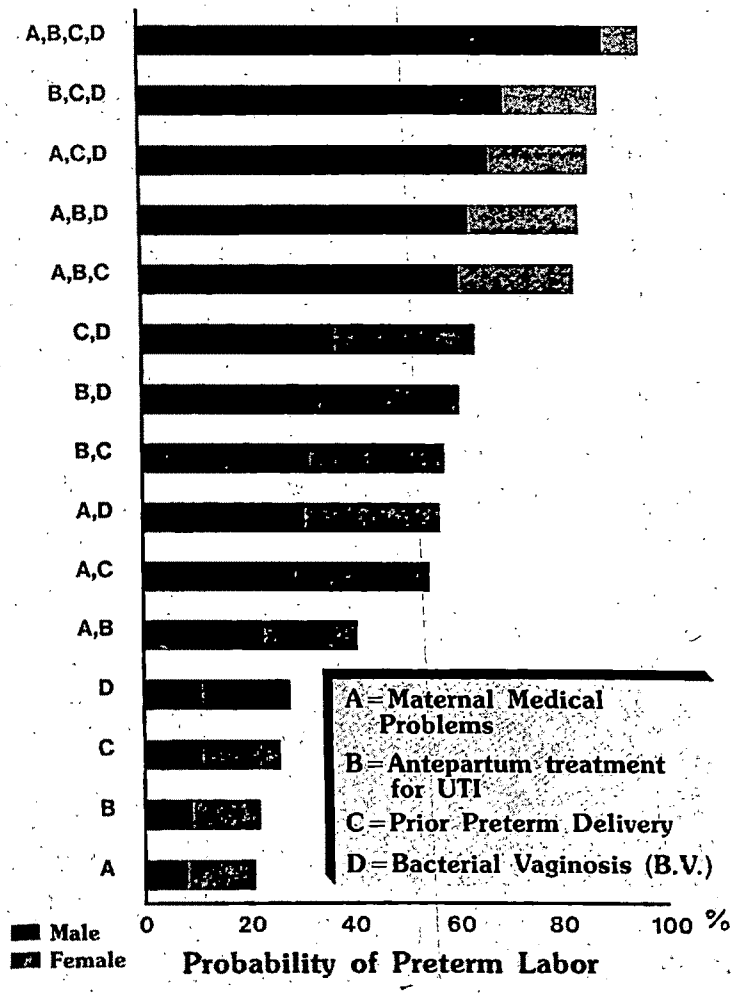


Fig. 1. Logistic regression analysis: Probability of preterm labor in presence of selected factors. (*UTI*, Urinary tract infection.)

term labor did not receive antimicrobials before the diagnosis.

To evaluate possible independent effects of several maternal historical factors and the presence of different microorganisms and conditions on the occurrence of preterm labor a logistic regression model was constructed. Maternal historical and microbiologic variables that approached significance ($p \leq 0.1$) with univariate analysis in this study or in past studies were incorporated into the model. Variables tested in the model were maternal medical problems, history of three or more abortions, maternal age, fetal gender, Creasy risk score, antepartum treatment for urinary tract infection, antepartum treatment with nontopical antimicrobial agents, recovery of *S. aureus, G. vaginalis, M. hominis, Mobiluncus species,* and the presence of bacterial vaginosis. Four factors remained in the model and were significantly related to development of preterm labor: prior preterm birth, maternal medical problems, antepartum treatment for urinary tract infection, and bacterial vaginosis (Fig. 1). In this cohort, women carrying female fetuses appeared to have increased risk of preterm labor compared with women carrying male fetuses. Among the single risk factors, the probability of preterm labor was highest for those women with bacterial vaginosis (males: RR, 4.6; females: RR, 11.3). Probability of preterm labor increased additively as more risk factors were included (Fig. 1). Calculations of RR, attributable risk and population-attributable risk for factors associated with preterm labor and birth (RR, ≥2.0; CI, not including 1.0) are shown in Table IV.

Comment

The incidence (4.5%) of preterm birth within this sample is lower than the 6% to 10% incidence usually observed in studies of similar U.S. populations.[3] This study's exclusion criteria, specific definition of preterm birth, and a possible unidentified selection bias may account for this relatively low occurrence of preterm birth. The low number (2%) of women in this study who had preterm PROM is consistent with similar populations.[3] Rates of recovery of lower reproductive tract

Table IV. Relative risk and attributable risk for factors associated* with preterm labor and preterm delivery

Pregnancy outcome	*Significant associations*	*Relative risk*	*Attributable risk*	*Population attributable risk (%)*
Preterm labor	Microbiologic associations			
	Bacterial vaginosis	2.6	0.62	23.0
	Staphylococcus aureus	3.1	0.68	5.9
	Mobiluncus species	3.8	0.74	14.1
	2 or more isolates or syndromes	3.3	0.70	48.8
	≥3 Abortions	2.9	0.66	11.5
	Maternal historic associations			
	Medical problems	2.4	0.58	17.4
	Prior preterm birth	3.6	0.72	12.2
Preterm delivery	*Mycoplasma hominis*	5.1	0.80	30.7
	2 or more isolates	6.9	0.86	71.2
	Prior preterm birth	6.7	0.85	37.9

*CI did not include 1.0 and RR ≥ 2.0.

microorganisms and recognition of bacterial vaginosis in this study are also consistent with findings in similar populations. Because of the relatively small number of women who had adverse outcomes and the large number of study factors analyzed, individual associations should be considered cautiously with confirmation sought in other studies.

Our findings support possible roles for bacterial vaginosis and associated microorganisms (i.e., *Mycoplasma hominis*) in preterm labor and birth. Past work is somewhat difficult to compare, principally because of differing criteria used for identification of bacterial vaginosis. Minkoff et al.[12] found an insignificant association between nonspecific vaginitis and preterm birth with a diagnosis based on presence of two of three clinical criteria. Subsequently, several investigators noted that clinical interpretation of vaginal findings for bacterial vaginosis is unreliable during pregnancy.[13, 20] In Minkoff's study,[12] *Bacteroides species* were the only identified microorganisms associated with increased incidence of preterm rupture of membranes and preterm birth among inner-city women. *Bacteroides species* are among the predominant anaerobic bacteria recovered from women with bacterial vaginosis.

In a case-controlled study comparing women in preterm labor and uncomplicated pregnancies, Gravett et al.[8] detected vaginal wash gas-liquid chromatographic profiles consistent with bacterial vaginosis significantly more often among the women with preterm labor. Subsequent work by these investigators with either gas-liquid chromatography or Gram stain to detect bacterial vaginosis further supports associations between bacterial vaginosis and preterm labor, preterm PROM, and amniotic fluid infection.[9, 13, 15]

As used here, vaginal Gram stain criteria have been shown to be reliable between trained observers and to more accurately predict positive clinical findings of bacterial vaginosis than either gas-liquid chromatography or vaginal culture for *G. vaginalis*.[20] Vaginal culture for *G. vaginalis*, even when done semiquantitatively, has been shown to be sensitive but nonspecific for detection of bacterial vaginosis.[20] *G. vaginalis* has been recovered from up to 80% of women without clinical evidence of bacterial vaginosis.[8] Therefore, it is not surprising that recovery of *G. vaginalis* in the present study was not associated with preterm labor or birth. Similarly, Gravett et al.[8] reported equivalent recovery rates for vaginal *G. vaginalis* among women admitted in preterm labor and matched control women with uncomplicated pregnancies.

The role of genital mycoplasmas in preterm birth remains controversial. This study confirms the work of others showing that antenatal isolation of *U. urealyticum* from either the vagina or endocervix is not associated with increased risk of prematurity.[11, 14, 30] In this study, 75% of the women who had preterm labor and 61% of those who did not experience preterm labor demonstrated vaginal *U. urealyticum*. In contrast, *U. urealyticum* recovered from between leaves of the amniochorion has been associated with histologic chorioamnionitis and preterm birth in several studies, including a well designed case-controlled study that statistically controlled for confounding obstetric factors.[7, 9] McCormack et al.[21] reported increased birth weight among *U. urealyticum*–colonized women who received antenatal erythromycin treatment in a double-blind placebo-controlled treatment trial investigating the role of genital mycoplasmas in pregnancy. This occurred even though treated women remained colonized. McCormack hypothesized that erythromycin treatment aids in prevention of upper genital tract colonization or infection with *U. urealyticum* during late gestation. However, the presence of other erythromycin-susceptible microorganisms such as

C. trachomatis, group B streptococcus, some *M. hominis* strains and anaerobes were not evaluated. Further, gestational length was not reported.

Although presence of *M. hominis* has been linked to prematurity and low birth weight, more recent work that controls for the presence of other bacteria or bacterial conditions has not consistently found *M. hominis* to be a significant risk factor for prematurity.[11, 12, 14] In this study, recovery of *M. hominis* was significantly associated with preterm birth. However, *M. hominis* was also significantly associated with the identification of bacterial vaginosis and *C. trachomatis*. *M. hominis* was not retained as an independent risk factor in our multivariate model for preterm labor.

C. trachomatis, group B streptococcus, and *S. aureus* were recovered from smaller numbers of the women in this study. *C. trachomatis* has been associated with increased risk of preterm birth when serologic evidence of recent or more extensive infection (immunoglobulin M) is present.[11, 14] Although serologic testing was not performed in this study, antenatal recovery of *C. trachomatis* in small numbers of patients (6.5%) was associated with increased risk ($p = 0.09$; RR, 4; 95% CI, 0.97 to 16.66) of preterm birth. Identification of group B steptococcus (3.5%) was not associated with poor obstetric or perinatal outcome, possibly due in part to the small number of women studied as well as antenatal and intrapartum treatment given to some culture-positive women. Although not commonly sought in women at risk of preterm birth, *S. aureus* has been recovered from the vagina in up to 5% of pregnant women.[22] We noted *S. aureus* in 3% of women sampled, with preterm labor occurring in half of these women (Table II).

The role of urinary tract infection and asymptomatic bacteriuria in preterm birth remains unclear.[23] Studies appear divided as to those that show a positive association between urinary tract infection and preterm birth and those that show no association. Both univariate and multiple regression analysis of our findings support antenatal urinary tract infection as a risk factor for preterm labor. That this effect occurs despite clinically adequate treatment suggests the presence of unrecognized associated factors or that urinary tract infection exerts a sustained deleterious effect.

Similar to the findings of Main et al.,[3] use of the standardized Creasy risk index for preterm birth in this population of poor women was not clinically useful in predicting those at risk. Fully 43% of women tested were designated as high risk for preterm birth by this tool; preterm labor actually developed in only 24% of these women and 7% were delivered of preterm infants. Indeed, one component of this scale, prior preterm birth, was a more accurate predictor of preterm labor than the overall risk index score.

Increased concentration of vaginal wash protease (≥10 trypsin units) was associated with increased risk of PROM. The association of increased vaginal wash protease activity with subsequent preterm rupture of membranes suggests that the presence of protease-releasing vaginal microorganisms or inflammatory responses to infection may lead to fetal membrane damage by spread through the cervix. Both bacteria and inflammatory cells can release proteolytic enzymes, including collagenases, which may weaken fetal membranes.[16, 24] Inflammatory mediators, including interleukin-1 and tumor necrosis factor, may initiate or potentiate these or other processes leading to preterm birth. Concentration of proteases and phospholipases and other factors are suggested as possible virulence factors for preterm birth and may originate from the cumulative presence of genital microorganisms and the host responses they engender.

Table IV shows calculated relative risks, attributable risks, and population-attributable risks for factors associated with preterm labor and preterm delivery. The presence of *S. aureus* in the mother's vagina was associated with greatest attributable risk of preterm labor. Inasmuch as recovery of *S. aureus* was uncommon (3%), the population-attributable risk was only 5.9%. Bacterial vaginosis was more common (18.7%) and associated with 23.0% population-attributable risk of preterm labor. Findings of *Mobiluncus species* morphotypes are also associated with a high calculated attributable risk of preterm labor. Whereas the presence of *Mobiluncus species* may constitute a separate risk factor, identification of mobiluncus may reflect increased severity of bacterial vaginosis.

History of either prior maternal preterm birth or ≥three abortions (spontaneous or induced) was also associated with increased attributable risk and population-attributable risk of preterm labor (Table IV). Prior preterm birth, but not prior abortion, has been consistently linked with subsequent preterm labor and birth in past studies.[4] Factors that account for increased risk of repeated preterm births remain unknown but could include ongoing subclinical endometrial infection, as suggested by Toth et al.[22] or some other persisting biologic predisposition.

Presence of *Mycoplasma hominis* was associated with 30% population-attributable risk of preterm birth. Again, whereas the presence of *M. hominis* might mediate specific risks of preterm birth, this microorganism occurred most commonly in the presence of bacterial vaginosis.

Interestingly, the presence of two or more microbial

infections or conditions was associated with significant risk of preterm birth (RR, 6.9; population-attributable risk, 71%). This suggests that whereas an individual infection or microbial condition may not have been powerful or frequent enough to be consistently associated with preterm labor or birth in this cohort, combinations of individual microbial conditions may contribute to a significant number of preterm births. Similarly, multivariate analysis (Fig. 1) suggests cumulative interactions occurring between presumably noninfection related factors, such as medical problems, and prior preterm birth with bacterial vaginosis.

Findings of this prospective study show that common and readily identifiable genital tract microbial infections or conditions are significantly associated with increased risks of preterm labor and birth. Whether selective treatment of one or more of these infections or conditions can effectively reduce occurrences of preterm birth is the subject of ongoing investigations.

REFERENCES

1. McCormack MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82-90.
2. Creasy RK, Gummer BA, Liggins GC. System for predicting spontaneous preterm birth. Obstet Gynecol 1980; 55:692-5.
3. Main DM, Richardson D, Gabbe SG, Strong S, Weller SC. Prospective evaluation of a risk scoring system for predicting preterm delivery in black inner city women. Obstet Gynecol 1987;69:61-6.
4. Iams JD, Johnson FF, Creasy RK. Prevention of preterm birth. Clin Obstet Gynecol 1988;31(3):599-615.
5. McGregor JA. Prevention of preterm birth: new initiatives based on microbial-host interactions. Obstet Gynecol Surv 1988;43(1):1-14.
6. Naeye RL, Blanc WA. Relation of poverty and race to antenatal infection. N Engl J Med 1970;283:555.
7. Kundsen RB, Driscoll SG, Monson RR, et al. Association of *Urealplasma urealyticum* in the placenta with perinatal morbidity and mortality. N Engl J Med 1984;310:941.
8. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986;67:229-37.
9. Hillier SL, Martius J, Krohn M, et al. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1988;319:972-8.
10. Cox SM, MacDonald PC, Casey L. Assay of bacterial endotoxin (lipopolysaccharide) in human amniotic fluid: potential usefulness in diagnosis and management of preterm labor. AM J OBSTET GYNECOL 1988;159:99-106.
11. Harrison HR, Alexander ER, Weinstein L, et al. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy. JAMA 1983;250:1721-7.
12. Minkoff H, Grunebaum AN, Schwarz RH, et al. Risk factors for prematurity and premature rupture of membranes: a prospective study of vaginal flora in pregnancy. AM J OBSTET GYNECOL 1984;150:965-72.
13. Gravett MG, Nelson P, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA 1986;256:1899-903.
14. Sweet RL, Landers DV, Walker C, Schachter J. *Chlamydia trachomatis* infection and pregnancy outcome. AM J OBSTET GYNECOL 1987;156:824-33.
15. Martius J, Krohn MA, Hillier SL, et al. Relationships of vaginal *Lactobacillus species*, cervical *Chlamydia trachomatis*, and bacterial vaginosis in preterm birth. Obstet Gynecol 1988;71:89-95.
16. McGregor JA, Lawellin D, Franco-Buff A, et al. Protease production by microorganisms associated with reproductive tract infection. AM J OBSTET GYNECOL 1986;154:109-14.
17. Lennette EH, Balows A, Hausler WJ Jr, Shadomy HG, eds. Manual of clinical microbiology, 4th ed. Washington, DC: American Society for Microbiologists, 1985.
18. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. J Clin Microbiol 1983;18:170-7.
19. Ross MG, Hobel CJ, Bragonier JR, Bear MB, Bemis RL. A simplified risk-scoring system for prematurity. Am J Perinatol 1986;3:339-44.
20. Krohn MA, Hillier SL, Eschenbach DA. Comparison of methods for diagnosing bacterial vaginosis among pregnant women. J Clin Microbial 1989;27:1266-71.
21. McCormack WM, Rosner B, Lee Y-H, et al. Effect on birth weight of erythromycin treatment of pregnant women. Obstet Gynecol 1987;69:202-7.
22. Toth M, Witkin SS, Ledger W, Thaler H. The role of infection in the etiology of preterm birth. Obstet Gynecol 1988;71:723-6.
23. Romero R, Oyarzun E, Mazor M, Sirtori M, Hobbins JC, Bracken M. Meta-analysis of the relationship between asymptomatic bacteriuria and preterm labor, delivery/low birthweight. Obstet Gynecol 1989;73:576-82.
24. Schoonmaker JN, Lawellin DW, Lunt B, McGregor JA. Bacteria and inflammatory cells reduce chorioamniotic membrane integrity and tensile strength. Obstet Gynecol 1989;74:590-6.

The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants

J. Studd,[a] M. Savvas,[a] N. Waston,[a] T. Garnett,[a] I. Fogelman,[b] and D. Cooper[c]
London, England

Twenty-three postmenopausal women with a median of 2 years past menopause (range, 1 to 12 years) and a median age of 52 years (range, 39 to 62 years) were recruited to participate in a longitudinal study designed to investigate the factors that influence the increase in bone density with subcutaneous estradiol and testosterone implants. All women received 75 mg of estradiol with 100 mg testosterone subcutaneously. Bone density was measured at the spine and hip by dual-photon absorptiometry before therapy and after 1 year of subcutaneous hormonal therapy. The mean pretreatment bone density at the lumber vertebrae and neck of the femur was 0.84 grams of hydroxyapatite per square centimer (SD, 0.11) and 0.73 grams of hydroxyapatite per square centimeter (SD, 0.10), respectively. The bone density at both sites increased with values of 0.91 grams of hydroxyapatite per square centimeter (SD, 0.11) and 0.75 grams of hydroxyapatite per square centimeter (SD, 0.11), respectively. These values represent an increase of 8.3% ($p < 0.0001$) at the spine and 2.8% ($p < 0.01$) at the neck of the femur. The plasma estradiol level increased from a median of 80.5 pmol/L to 453 pmol/L ($p < 0.001$). The percentage increase of vertebral bone density was not related to age, number of years past the menopause, pretreatment bone density, or serum testosterone levels, but a significant correlation was found between the percentage increase in bone density at the spine and the serum estradiol level ($p < 0.02$, $r = 0.45$). (AM J OBSTET GYNECOL 1990;163:1474-9.)

Key words: Osteoporosis, menopause, estradiol

After Albright's[1] observation that estrogen therapy can reduce urinary calcium excretion in postmenopausal women a number of prospective studies confirmed the values of estrogen replacement therapy in the prevention of postmenopausal osteoporosis.[2-4] Epidemiologic studies[5, 6] also showed a reduction in the incidence of osteoporotic fractures with such therapy.

Most studies used oral estrogen therapy, which although effective in the suppression of climacteric symptoms usually results in plasma estradiol and follicle-stimulating hormone (FSH) levels that are still in the postmenopausal range.[7] Although it has been claimed that the minimum dose of oral estrogen required to prevent postmenopausal osteoporosis is 0.625 mg conjugated equine estrogen[8] the optimal dose and route of estrogen necessary to achieve an increase in bone density is not known.

The percutaneous route of administration avoids the enterohepatic circulation and is associated with physiologic plasma levels of estradiol and estrone. This is in contrast to the low levels of estradiol and high levels of estrone found after oral therapy with both conjugated equine estrogens or estradiol valerate.[9, 10] Subcutaneous implants of estradiol and testosterone are effective in the alleviation of climacteric symptoms,[11] and a cross-sectional study[12] showed an apparent superiority of implant therapy over oral therapy in the therapeutic effect on bone density. The suggestion was made in this study that the greater bone density after implant therapy was a result of the greater plasma estradiol levels achieved with this route when compared with oral estrogen therapy.

We present the results of a prospective study of estradiol and testosterone implants on the bone density and plasma hormone levels in postmenopausal women over 1 year of therapy.

Patients and methods

A total of 23 postmenopausal women with a median age of 52 years (range, 39 to 62) were recruited into

From the Dulwich Hospital Menopause Clinic, Dulwich Hospital and Fertility and Endocrine Centre, Lister Hospital,[a] the Department of Nuclear Medicine, Guy's Hospital,[b] and the Computer Unit, King's College School of Medicine and Dentistry.[c]
Received for publication March 26, 1990; revised July 16, 1990; accepted July 25, 1990.
Reprint requests: John Studd, MD, Dulwich Hospital, East Dulwich Grove, London, England SE 22 8PT.
6/1/24109

Table I. Mean values of bone density at lumbar spine and neck of femur, body weight, blood pressure, and plasma hormone values before therapy and after 1 year of estradiol and testosterone implants

Treatment	*Pretreatment*	*1 Year after treatment*
Mean bone density at spine gHa/cm² (SD)	0.84 (0.11)	0.91 (0.11)*
Mean bone density at femur gHa/cm² (SD)	0.73 (0.10)	0.75 (0.11)†
FSH (IU/L) median (range)	71 (28-100)	12 (1-62)‡
Estradiol (pmol/L) median (range)	80.5 (30-580)	453 (204-883)‡
Testosterone (nmol/L) median (range)	0.6 (0.3-1.8)	0.9 (0.4-2.4)§
Mean weight in kg (SD)	67.3 (7.7)	66.7 (7.0)
Mean blood presure (mm Hg) (SD)	120 (37)/73 (12)	125 (21)/75 (13)

*$p < 0.0001$, Student's paired t test.
†$p < 0.01$, Student's paired t test.
‡$p < 0.001$, Wilcoxon matched-pair signed-rank test.
§$p < 0.01$, Wilcoxon matched-pair signed-rank test.

this prospective study. The median number of years past the menopause was 2 (range, 1 to 12). Menopausal status was defined as amenorrhea >1 year's duration with a serum FSH level >20 IU/L.

All women received hormone replacement therapy by subcutaneous implants of 75 mg estradiol and 100 mg testosterone. A further implant of the same dose of estradiol and testosterone was given after 6 months and again at 1 year.

Assays of FSH, estradiol, and testosterone were performed before treatment and at 1 year. The bone density was estimated in the spine of L2-4 and in the neck of the right femur with a Novo 22A BMC-LAB with gadolinium 153 as the source of radiation. The absorptiometer was standardized with a solution radiologically equivalent to hydroxyapatite, and the results were expressed as grams of hydroxyapatite per unit projected area of bone in square centimeters (gHa/cm²). The precision of the machine with the use of the phantom was 0.69% and short-term precision for normal subjects was 2.04%. Measurements were made before insertion of the hormone implant and at 12 months after therapy within 1 week of the insertion of the third implant.

Statistical analysis. The bone density values before and after treatment approximated to a normal distribution and the increase was therefore analyzed with the paired Student t test. The hormonal parameters could not be regarded as normally distributed and the changes were analyzed with the Wilcoxon matched-pairs signed-rank test. The Spearman correlation coefficient was calculated to express the relationship between the changes in bone density and the serum estradiol levels, serum testosterone levels, age, number of years past the menopause, and pretreatment bone density.

Results

Of the 23 patients studied 22 patients showed an increase in the bone density after 1 year of therapy (Table I). The mean bone density at the lumbar spine before therapy was 0.84 gHA/cm² (SD 0.11), which increased to 0.91 gHA/cm² (SD, 0.11) after 1 year ($p < 0.0001$). The value for the proximal femur was 0.73 gHA/cm² (SD, 0.10), which increased to 0.75 gHA/cm² (SD, 0.11, $p < 0.01$). The mean increase in the lumbar spine was 0.07 gHa/cm² (95% confidence interval, 0.06 to 0.08 gHa/cm²). The mean increase at the femur was 0.02 gHa/cm² (95% confidence interval, 0.01 to 0.04 gHa/cm²). These values represent a mean increase in the bone density of 8.3% at the spine and 2.8% at the neck of the femur after 1 year.

The median serum FSH level before therapy was 71 IU/L (range, 28 to 100 IU/L) with a median serum estradiol level of 80.5 pmol/L (range, 30 to 580 pmol/L) and a serum testosterone level of 0.6 nmol/L (range, 0.3 to 1.8). After 1 year of therapy the serum FSH level significantly reduced to 12 IU/L (range, 1 to 62; $p < 0.001$), the serum estradiol level increased to 453 pmol/L (range, 204 to 883; $p < 0.001$), and the serum testosterone level increased to 0.9 nmol/L (range, 0.4 to 2.4; $p < 0.01$).

A significant correlation was found between the percentage increase of vertebral bone density and the

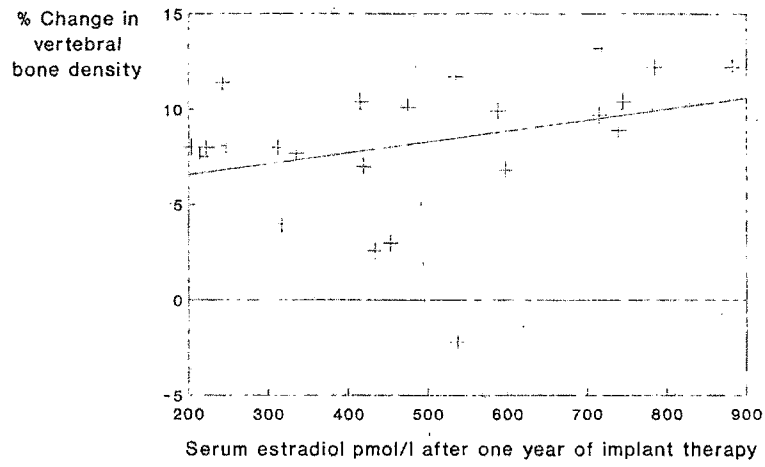


Fig. 1. Correlation between percentage increase in vertebral bone density with plasma estradiol levels after 1 year of therapy with estradiol and testosterone implants.

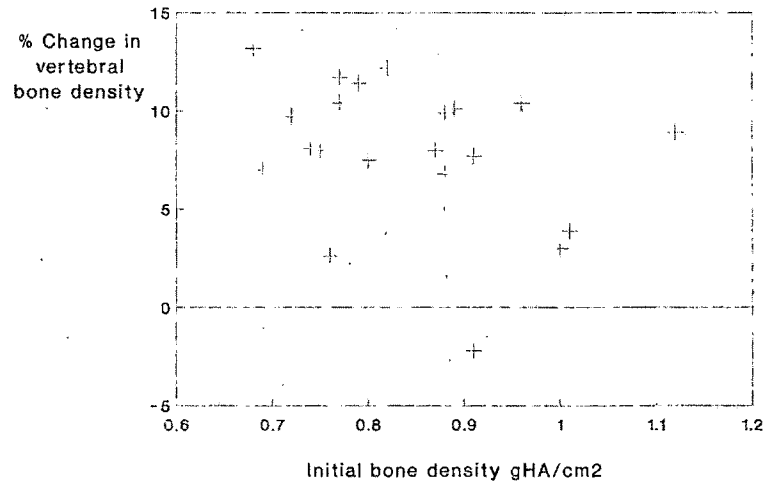


Fig. 2. Effect of pretreatment bone density on percentage increase in bone density with estradiol and testosterone implants.

plasma estradiol levels achieved after 1 year of therapy(Fig. 1, $r = 0.45$; $p < 0.02$). There was no significant correlation between the increase in bone density and the initial bone density (Fig. 2), age (Fig. 3), the number of years past the menopause (Fig. 4), and the serum testosterone level (Fig. 5).

Comment

These data show an increase of 8.3% in the bone density at the spine and 2.8% at the hip after 1 year of therapy with subcutaneous estradiol and testosterone implants. Evidence from our cross-sectional study with the use of the same method of bone densitometry reporting the vertebral bone density of 1.02 gHa/cm^2 and proximal femur values of 0.80 gHa/cm^2 after 8 years of such therapy[12] strongly suggest that this increase is not transient and will be maintained over the years.

Christiansen et al.[13] reported that the use of a combined preparation of oral estradiol, estriol, and norethindrone within 3 years of menopause resulted in an increase in bone density of 3% after 3 years of therapy. Lindsay et al.[14] (1984) found an increase of 2% to 4% over 5 years when mestranol 25 μg was prescribed within 3 years of menopause but this dose only maintained bone density if it was started more than 3 years after menopause. More recently Munk-Jensen et al.[15] reported an increase of 6.4% in the vertebral bone density of women treated within 2 years of menopause with continuous oral estradiol and norethindrone for 1 year. None of these authors reported the estradiol levels obtained with the therapy. In our study we were able to show an increase in bone mass even 10 years after menopause. This increase was the same as found in the younger postmenopausal woman and was also inde-

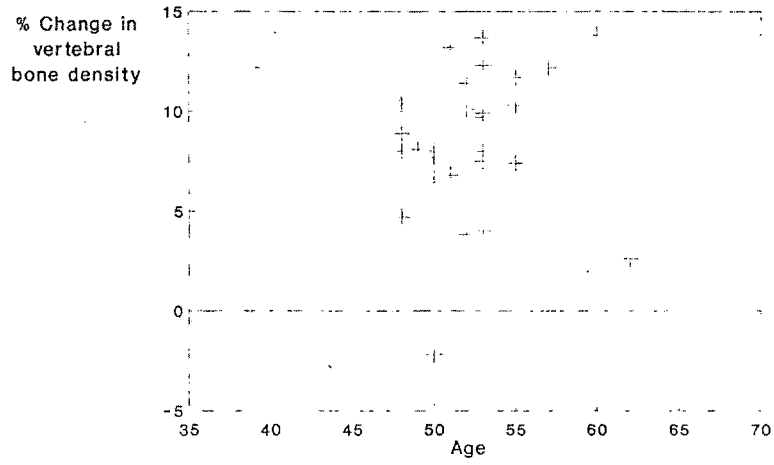


Fig. 3. Effect of age on percentage increase in bone density with estradiol and testosterone implants.

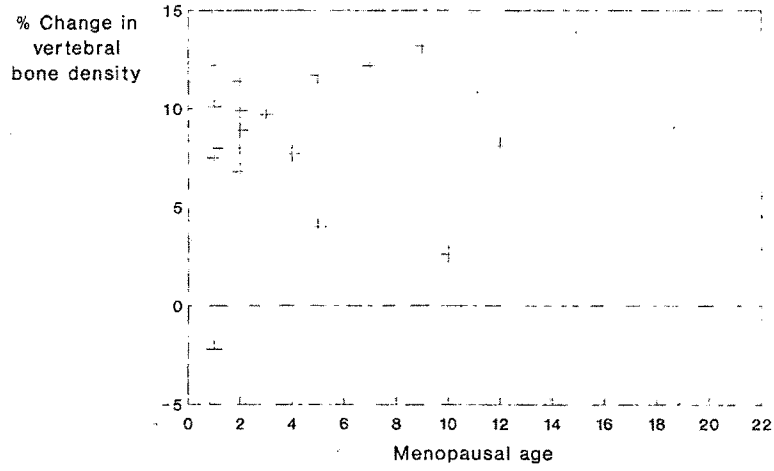


Fig. 4. Effect of number of years past menopause on percentage increase in bone density with estradiol and testosterone implants.

pendent of the initial bone density. The correlation between the percentage increase in bone density and the serum estradiol levels supports the hypothesis that the greater effectiveness of this mode of therapy may be a result of the higher serum estradiol levels achieved. Oral estrogen therapy is associated with lower estradiol levels and a less good skeletal response.[12]

The importance of the testosterone component of the treatment is unknown but there was no correlation in this study between plasma testosterone levels and the increase in bone density. Barlow et al.[16] could not show that the addition of testosterone to estrogen therapy improved bone density. However there is some evidence that adrenal androgens may have a role in maintaining the bone mass in postmenopausal women in the study of postmenopausal women with treated Addison's disease.[17] It is thus possible that the anabolic effects of testosterone on the skeleton may partly explain these results and this component of the therapy is subject to further investigation.

The increase in bone density was greater in the spine than that at the neck of the femur because the more active trabecular bone predominates in the vertebra.[18] There are several models that show such substantial reversal of bone loss, particularly in the vertebra. Treasure et al.[19] showed in a cross-sectional study that the bone loss of anorexia nervosa is reversed when menstruation returns. Greenspan[20] showed that the osteoporosis of hyperprolactinemic men with hypogondism recovers when the hyperprolactinemia is treated. Matta et al. (1988)[21] reported that the 5.9% loss of bone density after the use of buserelin for 6 months is reversed when it is discontinued. These conditions are all characterized by hypogonadism and the studies indicate

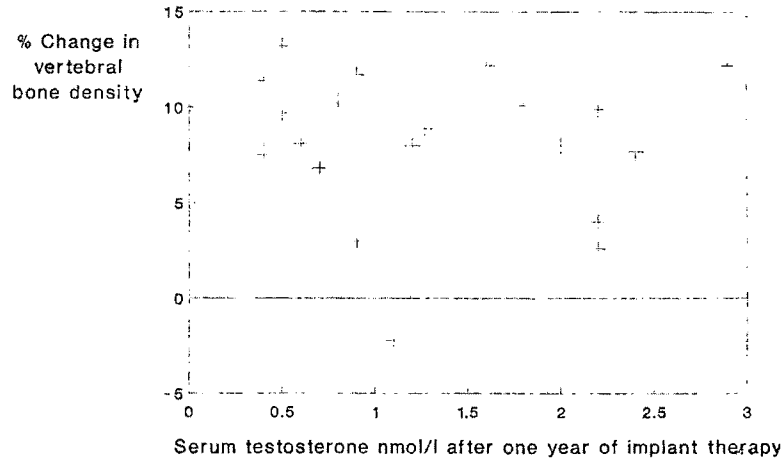


Fig. 5. Effect of serum testosterone after 1 year of therapy on percentage increase in bone density at spine.

that bone can be replaced in both men and women when plasma sex hormones return to normal physiologic levels.

The current recommended oral dosage of estrogens will suppress menopausal symptoms and prevent further bone loss but the serum estradiol and FSH levels achieved often remain in the postmenopausal range.[12] The dosage of orally administered estrogen may be limited by gastrointestinal symptoms and the liver impact on estrogen metabolism. It is probable that the safest way to achieve serum estradiol levels adequate to produce significant increases in bone mass is by the percutaneous route with either transdermal patches or subcutaneous implants.

The mechanism by which estrogen deficiency causes loss of bone remains unclear but the original Albright hypothesis of an association with a generalized loss of collagen including the collagenous matrix of the bone and the collagen of the skin is relevant. The substantial increase in vertebral spine density shown in this study can be compared with the 30% increase in skin collagen and a 25% increase in skin thickness that occurs in postmenopausal women who receive percutaneous estradiol therapy.[23] Only serial histomorphometric studies of bone biopsy specimens in these patients will reveal whether the estrogen-promoted changes in skin collagen are reproduced in the collagenous matrix of postmenopausal women.

It is reassuring from this study that estradiol and testosterone implants may not only prevent osteoporosis but will also be valuable in the older woman who might believe that it is too late to commence estrogen therapy. This therapy is also appropriate for the younger woman with premature ovarian failure who has already suffered substantial bone loss.

We gratefully acknowledge the assistance of Dereck Lowe, medical statistician, King's College Hospital, with the statistical analysis.

REFERENCES

1. Albright F, Smith PH, Richardson AM. Postmenopausal osteoporosis; its clinical features. JAMA 1941;116:2465-74.
2. Lindsay R, Hart DM, MacLean A, et al. Pathogenesis and of postmenopausal osteoporosis. In: Cooke ID, ed. The role of estrogen/progestogen management of the menopause. M. Lancaster, 1978:9-25.
3. Natchigall LE, Natchigall RH, Natchigall RD, Bechman E. Estrogen replacement therapy: a 10-year prospective study in the relationship to osteoporosis. Obstet Gynecol 1979;53:277-81.
4. Christiansen C, Christiensen MS, McNair P, Hagen C, Stocklund K, Transbol I. Prevention of early postmenopausal bone loss: controlled 2-year study in 315 normal females. Eur J Clin Invest 1980;10:273-9.
5. Ross RK, Panganini-Hill A, Mack TM. Reduction in fractures and other effects of estrogen replacement therapy in human population. In: Christiansen C, ed. Proceedings of the International Symposium on Osteoporosis 1984; 1:289-97.
6. Keil DP, Felson DT, Anderson JJ, Wilson DWF, Moskowitz MA. Hip fractures and the use of estrogens in postmenopausal women. N Engl J Med 1987;317:1169-74.
7. Chetkowski RJ, Meldrum DR, Steingold KA, et al. Biologic effects of transdermal estradiol. N Engl J Med 1986;314:1615-20.
8. Lindsay R, Hart DM, Clark HD. The minimum effective dose of estrogen for prevention of postmenopausal bone loss. Obstet Gynecol 1984;63:759-63.
9. Thom M, Collins WP, Studd JWW. Hormonal profiles in post-menopausal women after therapy with subcutaneous implants. Br J Obstet Gynaecol 1981;88:426-33.
10. Powers MS, Schenkel L, Darey PE, Good WR, Balestra JC, Place VA. Pharmacokinetics and pharmacodynamics of transdermal forms of 17β-estradiol: a comparison with conventional oral estrogens used for hormone replacement therapy. Am J Obstet Gynecol 1985;152:1099-106.
11. Cardozo ID, Gibb DMF, Studd JWW, Tuck SM, Thom MH, Cooper DJ. The use of hormone implants for cli-

macteric symptoms. AM J OBSTET GYNECOL 1984;148: 336-7.
12. Savvas M, Studd JWW, Fogelman I, Dooley M, Montgomery J, Murby B. Skeletal effects of oral estrogen compared with subcutaneous estrogen and testosterone in postmenopausal women. Br Med J 1988;297:331-3.
13. Christiansen C, Christiansen MS, Transbol I. Bone mass in postmenopausal women after withdrawal of estrogen/gestagen replacement therapy. Lancet 1981;1: 459-61.
14. Lindsay R, Hart DM, Aitken JM, MacDonald EB, Anderson JB, Clare AC. Long-term prevention of postmenopausal osteoporosis by estrogen. Lancet 1976;1: 1038-40.
15. Munk-Jensen N, Pors Nielsen S, Obel EB, Bonne Eriksen P. Reversal of postmenopausal vertebral bone loss by estrogen and progestogen: a double-blind placebo controlled study. Br Med J 1988;296:1150-2.
16. Barlow EH, Abdalla HI, Roberts ADG, Al Azzawi F, Leggate I, Hart DM. Long-term hormone implant therapy—hormonal and clinical effects. Obstet Gynecol 1986; 67:321-5.
17. Devogelaer JP, Crabbe J, Nagant De Deuxchainsnes C. Bone mineral density in Addison's disease: evidence for an effect of adrenal androgens on bone mass. Br Med J 1986;294:798-800.
18. Smith R. Recent advances in the metabolism and physiology of bone. In: Baker PF, ed. Recent advances in physiology, vol 10. Edinburgh: Churchill Livingstone, 1984: 317-48.
19. Treasure J, Russell GFM, Fogelman I, Murby B. Reversible bone loss in anorexia nervosa. Br Med J 1987; 295:474-5.
20. Greenspan SL, Neer RM, Ridgway EL, Klibanski A. Osteoporosis in men with hyperprolactinaemic hypogonadism. Ann Intern Med 1986;104:777-82.
21. Matta WH, Shaw RW, Hesp R, Evans R. Reversible trabecular bone density loss following GnRH analaogue, Buserelin in premenopausal women. Clin Endocrinol 1988;29:45-51.
22. Studd JWW, Anderson HM, Montgomery JC. Hormone treatment selection of patients—kind and duration of treatment. In: Greenblatt RB, ed. A modern approach to the menopause. Berlin: de Gruyter, 1986;182-98.
23. Brincat M, Kabalan S, Studd JWW, et al. A study of the relationship of skin collagen content, skin thickness and bone mass in the post menopausal woman. Obstet Gynecol 1987:840-5.

Fetal eye movement assessed with real-time ultrasonography: Are there rapid and slow eye movements?

Naoki Horimoto, MD, Takashi Koyanagi, MD, Shoji Satoh, MD, Toshiyuki Yoshizato, MD, and Hitoo Nakano, MD

Fukuoka, Japan

The goal of this study was to determine whether rapid eye movement and slow eye movement exist during the eye-movement period in the human fetus in utero. We studied 21 fetuses with real-time ultrasonography, 10 from 33 to 36 weeks and 11 from 37 to 41 weeks' gestation. We used the duration of eye-movement unit as a parameter and calculated the cumulative duration from the shortest to a given duration of eye movement per individual case. A scattergram of cumulative duration versus given duration obtained from all cases in each age group was analyzed with piecewise linear regression to search for a critical point(s). Critical given duration points were noted and reached statistical significance at 0.62 second and at 0.76 second during 33 to 36 and 37 to 40 weeks of gestation, respectively. These findings reveal two different types of eye movement: one with a duration of <0.6 to 0.8 second and the other with a duration of >0.6 to 0.8 second. These findings are compatible with previous criteria on rapid and slow eye movements, respectively, at 33 weeks of gestation onward. The mean value of cumulative duration at the critical point increased from 29.0% between 33 and 36 weeks to 47.1% between 37 and 41 weeks of gestation, indicating an increase in the proportionate amount of time maintaining rapid eye movement as gestation advances. (AM J OBSTET GYNECOL 1990;163:1480-4.)

Key words: Rapid eye movement, slow eye movement, human fetus, real-time ultrasonography

In 1955 Aserinsky and Kleitman[1] discovered two types of eye movements during sleep in the adult: rapid eye movements, which are characterized by rapid and jerky motions of relatively short arc, and slow eye movements, which are slow and lateral movements.

Studies have been made on eye-movement patterns in the developing human fetus by assessment with real-time ultrasonography.[2-5] However, it remains to be elucidated whether rapid-eye-movement sleep, non-rapid-eye-movement sleep, and waking states actually exist in the human fetus in utero.[6]

Slow eye movement was reported to reflect muscle hypotonia.[1] Therefore the coexistence of rapid and slow eye movements is a minimum requirement for identification of rapid-eye-movement sleep in the human fetus, as already evidenced in the neonate.[7]

From the Department of Gynecology and Obstetrics, Faculty of Medicine, Kyushu University.
Supported by a Grant-in-Aid for Scientific Research (no. 63480369), Japan.
Presented in part at the Second World Symposium, "Computers in the Care of the Mother, Fetus and Newborn," Kyoto, Japan, October 23-26, 1989.
Received for publication April 3, 1990; revised July 6, 1990; accepted July 16, 1990.
Reprint requests: Takashi Koyanagi, MD, Department of Gynecology and Obstetrics, Faculty of Medicine, Kyushu University 60, Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan.
6/1/23822

Thus in this study we attempted to assess whether the two types of eye movements exist during the eye movement period in the human fetus.

Material and methods

Fetal population. Twenty-one fetuses were randomly sampled. The fetuses were divided into two age groups: 10 from 33 to 36 weeks and 11 from 37 to 41 weeks of gestation.

The week of gestation was calculated from the last menstrual period and subsequently confirmed by routine ultrasonographic examination of fetal crown-rump length at 9 to 11 weeks' gestation.[8] All fetuses were found to be within the appropriate range of birth weight for each gestational week by Japanese standards (mean ± 1.5 SD)[9] with postnatal 1-minute Apgar scores of ≥8.

All mothers were cared for in the Maternity and Perinatal Care Unit, Kyushu University Hospital, and gave informed consent to participate in the study.

Data acquisition. A real-time ultrasonography scanner (model SSA-270A, Toshiba, Tokyo) with a convex transducer of 3.75 MHz was used. With the mother in the semirecumbent position, the observational standard plane was determined to be a nearly horizontal transection of the fetal head, yielding a clear image of the fetal lens in an orbit closest to the maternal abdominal wall (Fig. 1). The lens could be identified in the form

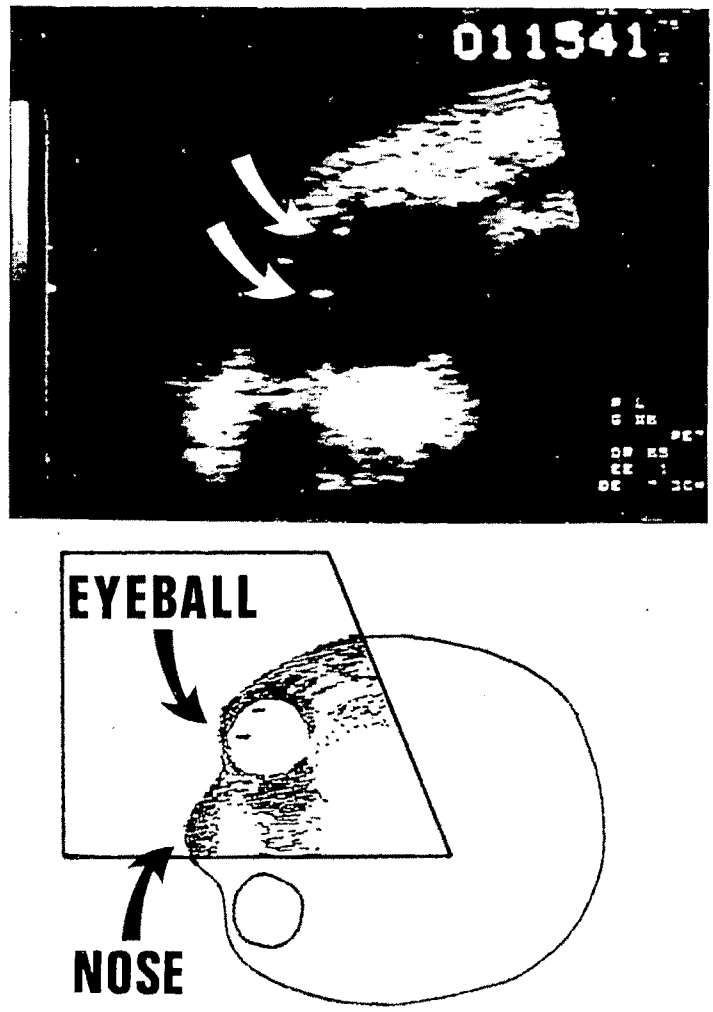


Fig. 1. Real-time ultrasonographic image for observation of fetal eye movement. *White arrows* indicate echoes originating from margins of lens. Number superimposed on right upper corner of this image indicates frame code.

of a pair of small dotted echoes, originating from both the near and the far margins of the lens, and was used as a landmark for quantifying eye movement.[2, 5] Each fetus was subjected to ultrasonographic examination only once for data acquisition in this study. An ultrasonographic image was continuously taken for 60 to 90 minutes and recorded by videotape recorder (model VO-9600, Sony, Tokyo) in real-time fashion. The frame code was simultaneously recorded on videotape with a frame-code generator (Sony model FCG-700).

Parameter definition and data processing. One unit of eye movement was defined as the movement of the lens from one stationary position through a position change to another stationary position. The eye movement period was regarded as the time sustaining the presence of movements for at least a 1-minute epoch, according to our previous reports,[5] and the eye movement period first noted after the start of the observation period was herein selected for analysis.

Data were processed on an individual case basis. While the videotapes were replayed, we determined the frame codes at the onset (F_{onset}) and the end (F_{end}) of each eye movement unit by use of slow motion and frame freezing. The duration (D) of the eye movement unit was then calculated in seconds as: $D = (F_{end} - F_{onset})/FR$, where FR is the frame rate of 29.97 frames per second.

After arranging all durations obtained in each fetus in ascending order [D_1 (shortest duration), D_2, D_3, ..., D_x (given duration), ..., D_N (longest duration)], we calculated the cumulative duration from the shortest to a given duration using the formula:

$$T_x = \left(\sum_{i=1}^{x} D_i / \sum_{i=1}^{N} D_i\right)$$

Statistical analysis. A scattergram of cumulative duration versus given duration (in logarithmic scale) was made of the data from all cases included in each age group. The scattergram was then analyzed by means

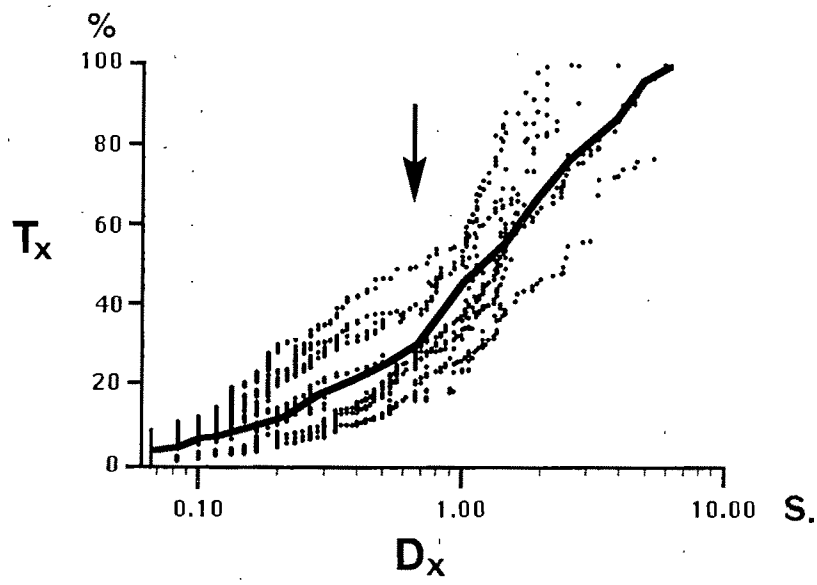


Fig. 2. Scattergram obtained from 10 cases included in 33- to 36-week gestational age group. Horizontal axis indicates duration of eye movement (D_x) in logarithmic scale, whereas vertical axis shows cumulative duration of every eye movement (T_x) by percent. *Solid line* indicates mean value of T_x. *Arrow* indicates statistically critical point.

Table I. Results obtained from piecewise linear regression analysis in both age groups

	p = 1		*p = 2*		*p = 3*	
Age group (wk)	*Critical points*	*Cp value*	*Critical points*	*Cp value*	*Critical points*	*Cp value*
33-36	0.62*	−4.53*	0.76, 1.58	−7.46	0.50, 0.89, 1.58	−6.21
	0.76	−3.04	0.76, 1.95	−7.44	0.38, 0.89, 1.58	−6.16
	0.89	−0.41	1.12, 11.58	−6.99	0.62, 0.89, 1.58	−6.14
37-41	0.76†	−8.20†	0.89, 1.95	−7.77	0.15, 0.89, 1.95	−6.63
	0.89	−7.96	0.89, 1.58	−7.49	0.18, 0.89, 1.95	−6.57
	0.62	−7.08	0.89, 2.51	−7.24	0.12, 0.89, 1.95	−6.34

p, Number of critical points; *Cp value*, Mallows' Cp value.
*Best critical point selected during 33 to 36 weeks' gestation.
†Best critical point selected during 37 to 41 weeks' gestation.

of piecewise linear regression to search for statistically critical given duration points. For selecting the best regression equation, Mallows' Cp value is defined as $Cp = RSS/s^2 - (n - 2p)$, where n is the number of fetuses, p is the number of critical points, RSS is the residual sum of the squares from a given combination of p points, and s^2 is the residual mean square based on the regression using all given duration points.[10] The procedure for selecting the best piecewise linear regression consisted of two steps. First, p was determined as the smallest p such that the Cp value is $\leq p$; then the best combination of critical points was selected as that when the smallest Cp value was found among the groupings of critical points.[11] All calculations were made with the computer package program, BMDP9R on an IBM system 4381 (Sumare, SP, Brazil).[12]

A simulation experiment for measuring eye-movement duration. To evaluate overall accuracy of the observation system, a simulation experiment was made apart from this study (data not shown) from data acquisition, through recording of the ultrasonographic image in videotapes, replaying the videotapes and determining the frame codes at the onset and the end of each eye movement, and finally by measuring the duration of each eye movement unit. As a model for the fetal eyeball, we used a mechanical sector scannerhead (ASU-32H-3.5, Aloka, Tokyo), in which the diameter of the discoid transducer element and the scanning range resembled the size of the fetal lens and the horizontal oculomotor range in adults,[13, 14] respectively. The movement of the sector scanner transducer, submerged into deaerated water, was observed with the convex scannerhead used in the main study and was

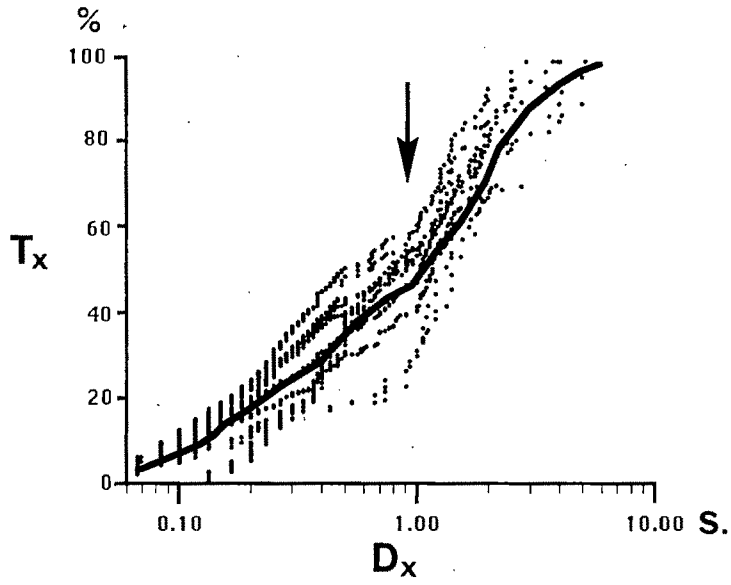


Fig. 3. Scattergram obtained from 11 cases in age group from 37 to 41 weeks' gestation. Display format and abbreviations used are described in Fig. 2.

recorded on videotape. This test was performed at two different cycles; 0.08 second and 0.09 second. Consequently, the mean values (± SE) for the two cycles were 0.08 ± 0.01 and 0.09 ± 0.01 second, and the coefficients of variation were found to be 12.5% and 11.1%, respectively.

Results

Fig. 2 shows the scattergram of the cumulative duration versus given duration in the first age group between 33 and 36 weeks' gestation. Given duration ranged from 0.07 to 4.75 seconds. One critical given duration point was indicated with statistical significance at 0.62 second (Cp = −4.53; Table I), at which the mean cumulative duration value was 29.0%.

Fig. 3 demonstrates the scattergram of cumulative duration versus given duration in the second age group at ≥37 weeks' gestation; given duration ranged from 0.07 to 5.20 seconds. One statistically critical given duration point was noted at 0.76 second (Cp = −8.20) among fetuses in this group (Table I). The mean cumulative duration value at the critical point was 47.1%.

Comment

We focused our study on fetuses at ≥33 weeks' gestation in consideration of our previous report that states that eye movements become consolidated and form a period of rapid eye movement or so-called rapid eye movement period by this point in gestation.[5] Rapid eye movement periods reach an almost uniform length of time and last approximately 30 to 40 minutes at 36 to 37 weeks of gestation onward. Taking into consideration the chronologic development of the rapid eye movement period according to advances in gestation, we divided fetuses into two age groups to document whether age-related change in the manifestation of rapid and slow eye movements is present between 33 to 36 weeks and 37 to 41 weeks of gestation.

Piecewise linear regression analysis is performed in any study to identify critical points, if any, without prior knowledge of their existence. Such analysis reveals that measurements with values ranging less than and more than critical points differ in characteristics, thus reflecting underlying factors.

From this study one critical point per age group was evident at 0.62 and 0.76 second in the duration of the eye movement unit during 33 to 36 weeks and 37 to 41 weeks of gestation, respectively, thereby indicating that two distinct kinds of eye movements exist in both age groups: one with durations from 0.07 to 0.6-0.8 second and the other with durations from 0.6-0.8 second to 4-5 seconds (Figs. 2 and 3). Our findings are compatible with those of Aserinsky and Kleitman,[1] who termed rapid and slow eye movement by the duration of such movement, in which the former lasts approximately 1 second and the latter lasts 3 to 4 seconds. Therefore the two types of eye movements we observed can be considered to correspond with the criteria of Aserinsky and Kleitman of rapid eye movement and slow eye movement. The age-related difference between 33 to 36 weeks and 37 to 41 weeks' gestation is seen by the increase in the proportionate amount of time maintaining rapid eye movement, as indicated by the increase in the cumulative duration of such movement at the critical points.

Because of the anatomic structure of the extraocular muscles attached to the orbital socket, in the absence of the supranuclear control necessary for extremely fine integration of neural activity, the extraocular muscles become hypotonic with incoordinate waxing and waning that results in the occurrence of slow eye movements.[1] Thus slow eye movements reflect decreased skeletal muscle tone, which represents the tonic phenomenon during rapid eye movement sleep, and its control mechanism differs from that of the phasic phenomenon, such as rapid eye movement. Studies have also reported that infants delivered at 33 to 35 weeks show a reduction in muscle action potentials during rapid eye movement sleep.[15, 16]

On the basis of previous reports, our findings from this study will further the determination of whether the rapid eye movement period can be linked, using rapid eye movement and slow eye movement as indicators, to rapid eye movement sleep in the human fetus in utero at 33 weeks' gestation and therafter.

We thank L. Saza for editorial support, K. Akazawa, Kyushu University, for statistical analysis, and T. Suzuki, Kyushu University, for computer assistance.

REFERENCES

1. Aserinsky E, Kleitman N. Two types of ocular motility occurring in sleep. J Appl Physiol 1955;8:1-10.
2. Birnholz JC. The development of human fetal eye movement patters. Science 1981;213:679-81.
3. Bots RSGM, Nijhuis JG, Martin CB Jr, Prechtl HFR. Human fetal eye movements: detection in utero by ultrasonography. Early Hum Dev 1981;5:87-94.
4. Horimoto N, Koyanagi T, Nagata S, Nakahara H, Nakano H. Concurrence of mouthing movement and rapid eye movement/non-rapid eye movement phases with advance in gestation of the human fetus. Am J Obstet Gynecol 1989;161:344-51.
5. Inoue M, Koyanagi T, Nakahara H, Hara K, Hori E, Nakano H. Functional development of human eye movement in utero assessed quantitatively with real-time ultrasound. Am J Obstet Gynecol 1986;155:170-4.
6. Aserinsky E. Eye movement patterns in infants. Science 1982;215:1274.
7. Prechtl HFR, Lenard HG. A study of eye movements in sleeping newborn infants. Brain Res 1967;5:477-93.
8. Sakamoto S, Okai T. Ultrasonic and endocrinological aspects of first trimester miscarriage. Asia Oceania J Obstet Gynaecol 1982;8:105-16.
9. Nishida H, Sakanoue M, Kurachi K, Asada A, Kubo S, Funakawa H. Fetal growth curve of Japanese. Acta Neonat Jpn 1984;20:90-7.
10. Draper N, Smith H. In: Applied regression analysis. 2nd ed. New York: John Wiley, 1981:294-379.
11. Nakamura T. BMDP program for piecewise linear regression. Comput Methods Programs Biomed 1986;23:53-5.
12. Dixon WHJ, Brown MB, Engleman L, et al. BMDP statistical software. Berkeley, California: University of California Press, 1985.
13. Jeanty P, Dramaix-Wilmet M, Van Gansbeke D, Van-Regemorter N, Rodesch F. Fetal ocular biometry by ultrasound. Radiology 1982;143:513-6.
14. Robinson PC. Control of eye movements. In: Brookhart JM, Mountcastle VB, Brooks VB, et al. eds. Handbook of physiology—the nervous system. II. Bethesda, Maryland: American Physiological Society, 1981:1275-320.
15. Parmalee AH, Stern E. Development of states in infants. In: Clemente CD, Purpura DP, Mayer FE, eds. Sleep and the maturating nervous system. New York: Academic Press, 1972;199-215.
16. Petre-Quadens O. Ontogenesis of paradoxical sleep in the human newborn. J Neurol Sci 1967;4:153-7.

Continuous or interrupted fascial closure: A prospective evaluation of No. 1 Maxon suture in 402 gynecologic procedures

James W. Orr, Jr., MD, Pamela F. Orr, RN, Jeffrey M. Barrett, MD, John R. Ellington, Jr., MD, Ralph H. Jennings, Jr., MD, Keith B. Paredes, MD, Bruce Patsner, MD, and Dale L. Taylor, MD

Lakeland, Florida

During a 14-month period of using a long-term absorbable suture (No. 1 Maxon), 402 patients were entered into a prospective, randomized trial of fascial closure. Patients were randomized between a continuous closure (201 patients) and an interrupted en bloc (201 patients) technique. Each patient was subjected to a preoperative and intraoperative protocol for wound management. There were no acute wound failures. Wound infection rates and risk of hernia were not apparently affected by closure technique. (AM J OBSTET GYNECOL 1990;163:1485-9.)

Key words: Abdominal closure, absorbable suture

Gynecologists currently perform over 4 million operative procedures yearly in this country, 75% of which involve an abdominal incision.[1] Incision type and placement are guided by numerous variables including diagnosis, urgency, patient build, the presence or absence of previous incisions, the need for a stoma, or cosmetic preferences. To date, no prospective, randomized data concerning wound closure after gynecologic procedures exists. Therefore the selection of closure technique and suture material is usually governed by anecdotal experience, familiarity, prejudice, and previous training.

This article details a prospective evaluation of the efficacy and benefits of a nonlocking continuous, en bloc closure versus an interrupted far-far/near-near (Smead-Jones) closure in 402 gynecologic incisions using a long-term, absorbable, polyglyconate suture.

Methods and material

During the 14 months before September 1988, 404 women were entered into the study protocol. Prior approval by the Institutional Review Board of Lakeland Regional Medical Center and the Watson Clinic, Lakeland, Florida had been obtained. Each patient provided written, informed consent. The patients' risk criteria (Table I) were evaluated, and patients were eligible if their total score was <7. After entry, a computer generated randomization table was used to allocate patients into a continuous or interrupted en bloc closure technique. There was no attempt to stratify patients as to incision type because this decision was made intraoperatively. All eligible patients who consented were included in this surgical protocol.

Every patient was evaluated preoperatively by at least one of the authors (P. O.) and was examined daily during the hospital stay by at least two of the authors; 1-month and 6-month evaluations were also performed.

Surgical preparation was relatively uniform. Preoperative hospitalization was minimized and the majority (54%) of patients were admitted the day of surgery. Preoperative mechanical bowel preparation was used in all patients with a known or suspected diagnosis of malignancy. No preoperative douche was performed, and the vagina was prepped only in the operating room. If hair removal was deemed necessary, it was clipped in the operating room. All incisions were at least 15 cm in length. Single-dose antibiotic prophylaxis was routinely used; no patient received more than three doses. All vaginal cuffs were closed with 2-0 polyglycolic acid sutures. The visceral or parietal peritoneum was not routinely closed. Copious pelvic irrigation was performed before closure. Closed suction pelvic drainage was used only in patients who had radical hysterectomy and bilateral pelvic lymphadenectomy.

Fascial closure incorporated No. 1 Maxon suture (Davis & Geck, Danbury, Conn.) in all patients. Inter-

From the Division of Gynecologic Oncology, Watson Clinic.
Presented at the Annual Meeting of the Society of Gynecologic Surgeons, New Orleans, Louisiana, February 6, 1990.
Received for publication December 27, 1989; revised June 25, 1990; accepted July 5, 1990.
Reprint requests: James W. Orr, Jr., MD, Division of Gynecologic Oncology, Watson Clinic, P. O. Box 95000, Lakeland, FL 33804-5000.
6/1/23624

Table I. Risk criteria—fascial closure

Risk factor	*Score*
Weight/height ratio (>120%)	2
Weight (>200 LBs)	1
Previous radiation	3
Cancer	2
Active infection	3
Malnutrition (albumin <3.0 gm/dl)	1
Steroids	1
Diabetes	2

Low risk <7.

rupted closures involved an en bloc far-far/near-near (Smead-Jones) technique in vertical incisions and a wide-placed tissue "bite" in transverse incisions.[2] When an interrupted closure was used, the fascial edge to suture interface was at least 1.5 cm and the suture-to-suture distance was 1.5 to 2.0 cm. The peritoneum was only incorporated into the closure if easily mobilized. Continuous closures involved a two-suture technique, either from side to side or above and below. Sutures were placed at least 1.5 cm from the fascial edge and 1.5 to 2.0 apart. All knots were secured with at least a surgeon's-surgeon's/square-square knot. Knots were secure but not tied so as to strangulate tissue. Copious irrigation of the subcutaneous tissues was usual. Subcutaneous sutures or drains were not used. Skin clips were the chosen method of skin closure. All incisions and closures were performed by board-certified gynecologists.

Results

During the study period, 404 patients were registered and 402 patients were evaluable; the two unevaluable patients underwent vaginal operation. Randomization allotted 201 patients into a continuous and 201 patients into an interrupted fascial closure. One hundred forty-eight patients (36.8%) had a transverse incision and 254 had a vertical incision. Review of the identifying data indicated that the patients could be delineated into two groups: those with a transverse incision and those with a vertical incision (Table II). The two incision groups differed in number of patients, mean age (t test; $p < 0.0001$), weight ($p = 0.0036$), and risk score ($p < 0.0001$). However, within incision type, there was no difference in these factors between those patients with a continuous and interrupted closure. These incision type differences also existed in the postoperative diagnosis (Table III) and the operative procedure (Table IV). As expected, the majority of procedures for surgical treatment of a suspected gynecologic cancer or known malignancy were performed through a vertical incision.

The majority of procedures were classified as clean or clean contaminated (Table V). Mean fascial closure time was statistically ($p < 0.01$) and clinically shorter in the continuous closure group regardless of incision type (Table VI).

Acute and long-term complications were rarely encountered in those patients with a transverse incision (Table VII). Although the overall incidence appeared increased in patients with vertical incisions, there was no statistical or clinical difference in the incidence of seroma or infection ($p = 0.10$). When the continuous and interrupted closures in this series were compared, the risk of hernia was not increased in the presence of infection, and the method of closure did not increase the risk ($p = 0.5$). The mean age and weight for those patients in whom a hernia developed was 59.6 years and 155 pounds. The mean risk score for those with hernias was 2.8 (continuous) and 3.0 (interrupted). Neither complete wound dehiscence nor evisceration developed in any patient.

The infection/seroma rate (2.68) was 2.1% in clean wounds; 3.1% (10/325) in clean contaminated wounds, and 22.1% (2/9) in contaminated wounds.

Comment

A predictable pattern of healing occurs after a surgical incision.[2] Surgical and natural hemostasis, an immediate requirement, is followed by the release of vasoactive substances, an influx of polymorphonuclear leukocytes, and a state of acute inflammation. After 3 days capillary proliferation begins at the wound edge and randomly oriented collagen monomers are found. Approximately 4 days later the phase of collagen maturation begins and the resultant wound stress and covalent collagen binding begins to impart strength to the wound. Even at this time (approximately 7 days), incisional strength remains totally related to suture strength.

Although the wound-breaking tension of a given incision may vary dramatically (with a fivefold to tenfold interpersonal difference), the abdominal fascia apparently regains its strength slowly. In fact, less than 40% of the unwounded tensile strength is present at 8 weeks.[3] The majority of known absorbable sutures actually retain only about 10% of their original tensile strength at 3 weeks[2] and potentially place the abdominal wound at risk for late complications such as hernia formation. Permanent sutures maintain constant tensile strength during this healing period. However, the use of a permanent suture increases the risk of late wound pain or sinus formation. In consideration of these facts, an absorbable suture with a slower loss of tensile strength could be of potential benefit by maintaining wound strength during the early phase of healing and not becoming a permanent foreign body with its inherent problems.

Fascial strength is also related to suture placement.

Table II. Fascial closure with No. 1 Maxon

	Transverse		*Vertical*	
	Continuous	*Interrupted*	*Continuous*	*Interrupted*
No. of patients	72	76	129	125
Age (yr)				
Mean	38.5 + 1.1	40.8 + 1.3	54.5 + 1.3	52.9 + 1.2
Median	36	38	53	53
Weight (lb)				
Mean	143.6 + 3.1	143.6 + 3.6	155.3 + 3.1	150.4 + 2.8
Median	138	137	146	144
Mean risk score	0.67 + 0.2	0.82 + 0.2	2.07 + 0.2	2.15 + 0.2
% Risk score >1	28.2	34.2	73	79.2

Table III. Postoperative diagnosis

	Transverse		*Vertical*	
Diagnosis	*Continuous*	*Interrupted*	*Continuous*	*Interrupted*
Leiomyomas	25.0	14.5	17.8	17.6
Endometriosis	15.3	13.2	4.7	4.0
Benign ovarian cyst	9.7	9.2	9.3	12.0
Hyperplasia	8.3	7.9	7.0	3.2
Cervical intraepithelial neoplasia	4.2	1.3	0.8	
Cervical cancer	9.7	13.2	5.4	8.8
Incontinence	9.7	13.2		4.0
Bleeding	4.2	7.9	4.7	1.6
Endometrial cancer		1.3	10.1	8.8
Ovarian cancer			12.4	20.0
Other	13.9	18.2	28.4	20.0
TOTAL	100.0	99.9	100.0	100.0

Table IV. Operative procedure

	Transverse		*Vertical*	
Procedure	*Continuous (%)*	*Interrupted (%)*	*Continuous (%)*	*Interrupted (%)*
Hysterectomy plus benign	65.3	61.8	45.0	45.6
Hysterectomy plus malignant			15.5	13.6
Radical hysterectomy	9.7	11.8	4.7	5.6
Exenteration		1.3		
Surgical reassessment or debulking			17.1	21.6
Other	25	25.1	17.7	19.8

Tera and Aberg[4] reported that wound closure strength can be doubled in vertical incisions if sutures are placed 1.5 to 2.0 cm away from the linea alba. These data have some clinical support because retrospective clinical studies indicate a low risk of fascial dehiscence in vertical incisions closed with an en bloc technique.[5] Unfortunately, the same clinical data are not available in the closure of transverse incisions because intermediate (7-day) and long-term (21-day) wound strength is not apparently increased with an en bloc closure.[6] Although the transverse incision is only half as strong as a vertical incision closed en bloc,[7] the postoperative stress placed on a transverse incision appears less, and clinically this cosmetic incision performs well. In fact, the clinical success of the transverse incision may not be related to its strength but rather to the bias of physicians to use this incision in young, healthy women.

Knot tying techniques may be as important in maintenance of incisional strength as suture placement. Tight knots potentially strangulate tissue and interfere with optimal wound healing.[6]

The study protocol was designed to control these variables and to evaluate the effects of different methods of wound closure with a single-suture material only.

Table V. Operative procedure

	Transverse		*Vertical*	
Type incision	*Continuous (%)*	*Interrupted (%)*	*Continuous (%)*	*Interrupted (%)*
Clean	13.9	17.1	16.3	19.2
Clean contaminated	86.1	79.0	81.4	78.4
Contaminated		3.9	2.3	2.4

Table VI. Fascial closure time

	Transverse		*Vertical*	
	Continuous	*Interrupted*	*Continuous*	*Interrupted*
Closure time (min)				
Mean	4.5	8.75	4.75	12.10
Range	2.75-7.0	5.5-11.0	3.0-6.5	6.5-14.5

Table VII. Complications of fascial closure

	Transverse		*Vertical*	
	Continuous (%)	*Interrupted (%)*	*Continuous (%)*	*Interrupted (%)*
Seroma/infection	2.6	0	5.6	3.2
Hernia				
1 mo	0	0	0	0
6 mo	0	0	3.9	2.4
Dehiscence	0	0	0	0

Few prior reports recognize and control for these potential confounding variables. As a result, gynecologists are required to place over 40 million fascial sutures each year, relying on general surgical data to make decisions. Unfortunately, these data are often biased by the inclusion of males and a different incision placement, both of which may alter the risk of wound outcome.[8]

Not uncommonly, previous prospective papers evaluated different suture composition, different suture size, and different closure techniques in a single protocol.[9, 10] The inclusion of these additional variables even in large studies makes it difficult to clarify the salient information. Wissing et al.[10] recently reported a prospective randomized multicenter study of fascial closure in 1400 patients that evaluated No. 2 polygalactin (interrupted), No. 1 polygalactin (continuous), No. 1 polydioxanone sulfate (continuous), and No. 1 nylon (continuous) sutures.[11] The study was conducted in multiple institutions, there was no stratification of wound risk factors, and there was no report of control of the preoperative or intraoperative factors important in wound healing. However, it apeared that the hernia rate (10.4% to 20.6%) of all reported closure techniques in this prospective study was significantly higher than reported in previous retrospective reviews. In fact, this prospective incidence was twentyfold higher than two recent retrospective reports in the gynecologic literature.[11, 12] In addition, chronic wound pain or wound sinus problems were common with use of permanent suture.[10]

In contrast to previous investigations, our protocol attempted to control for factors that predispose to wound infection since all incisions were closed by experienced gynecologists, patients had a short hospitalization, hair clipping, and short-term antibiotic prophylaxis. Although not usually stated in previous reports, even the last factor may be important because recent data suggest that longer-term prophylaxis may adversely affect wound healing and result in late wound failures.[13] Small (2-0) polyglycolic acid sutures were used during the pelvic portion of the operation. Use of subcutaneous sutures, which potentially increase the risk of infection, and subcutaneous drains were avoided. Each incision was closed with widely placed sutures to approach an optimal suture length to wound length ratio of 4:1.

Our protocol evaluated a polyglyconate suture [32% trimethylene carbonate (pliability) and 68.1% polyglycolic acid copolymer (strength)] that maintains as much as 40% of its tensile strength at 6 weeks.[14] The hydrolysis-associated breakdown allows more gradual

loss of suture strength and theoretically mirrors the return of strength of the healing fascia. The monofilament character of this suture allows ease of passage through tissue and may decrease the risk of infection when compared with braided materials. Surgeon's knots were used because they impart the greatest strength to a suture. Finally, some preoperative attempt to stratify for risk of poor wound outcome was used. Although the risk categorization may at first appear arbitrary, the authors desired that no single risk factor would eliminate a patient from the protocol. Although it might appear that these patients were "low" risk, the mean risk scores suggest that those patients with vertical incisions had a significant incidence of factors that might adversely affect wound outcome.

Our data suggest that wound infection or seroma risks were not affected by the method of fascial closure with use of a strict wound management protocol and a monofilament suture. However, it was interesting that late postoperative seromas (>10 days) developed in 16 patients during the protocol. The risk of incisional hernia was not affected by the method of closure.

The results of our study suggest that in a private practice of gynecology, wound closure by a continuous closure technique with a long-acting absorbable suture appears to be associated with few short-term and long-term wound problems when compared with an en bloc closure. This closure may be performed in less than half the operative time and apparently does not increase the risk of long-term wound complications. The importance of shortening the operative procedure has numerous benefits, both in cost savings and decreasing infectious risks, particularly in those procedures of intermediate operating time when closure may account for as much as 20% of the operative time.

REFERENCES

1. Rutkow IM. Rates of surgery in the United States: the decade of the 1970s. Surg Clin North Am 1982;62:559-78.
2. Orr JW Jr. Sutures and closures. Ala J Med Sci 1986;23:36-41.
3. Lichtenstein IL, Herzikoff S, Shore JM, et al. The dynamics of wound healing. Surg Gynecol Obstet 1970;130:685-90.
4. Tera H, Aberg C. Tissue strength of sutures involved in musculoaponeurotic layer sutures in laparotomy incisions. Acta Chir Scand 1976;142:349-55.
5. Wallace D, Hernandez W, Schlaerth JB. Prevention of abdominal wound disruption utilizing the Smead-Jones closure technique. Obstet Gynecol 1980;56:226-30.
6. Poole GV. Mechanical factors in abdominal wound closure: the prevention of fascial dehiscence. Surgery 1985;97:631-9.
7. Tera H, Aberg C. The strength of suture knots after one week in Rievo. Acta Chir Scand 1976;142:301-7.
8. Ellis H, Bucknall TE, Cox PJ. Abdominal incisions and their closure. Curr Prob Surg 1985;22:5-51.
9. Sauolarnen H, Ristkari S, Mokka R. Early laparotomy wound dehiscence: a randomized comparison of three suture materials and two methods of fascial closure. Ann Chir Gynaecol 1988;77:111-3.
10. Wissing J, Van Vroonhoven T, Eeftinck M, et al. Fascial closure after midline laparotomy: results of a randomized trial. Br J Surg 1987;74:738-41.
11. Sheppard J, Cavanaugh D, Riggs D, et al. Abdominal wound closure using a nonabsorbable single layer technique. Obstet Gynecol 1983;61:248-52.
12. Gallup D, Talledo O, King L. Primary mass closure of midline incisions with a continuous monofilament suture in gynecologic patients. Obstet Gynecol 1989;73:675-7.
13. Scher KS, Scott-Conner CEH, Montany PF. Effect of cephalosporins on fascial healing after celiotomy. Am J Surg 1988;155:361-5.
14. Foresman PA, Edlich RF, Rodeheaver GT. The effect of new monofilament absorbable sutures on the healing of musculoaponeurotic incisions, gastrotomies and colonic anastomoses. Arch Surg 1989;124:708-10.

Endometrioma of the liver

Vittorio Rovati, MD,[a] Edoardo Faleschini, MD,[a] Paolo Vercellini, MD,[b] Giovanni Nervetti, MD,[a] Giorgio Tagliabue, MD,[a] and Guido Benzi, MD[b]

Milan, Italy

Hepatic endometriosis is extremely rare. We describe a patient sent to us with epigastric pain as the only symptom and who was found to have associated endometrioma of the liver and left ovary. We suggest a gynecologic evaluation before surgery for hepatic cyst of unknown cause. (Am J Obstet Gynecol 1990;163:1490-2.)

Key words: Endometriosis, liver cyst, abdominal pain

The occurrence of extrapelvic endometriosis is not uncommon. The only organ in the abdominal cavity that is apparently refractory to the disease is the spleen.[1] We are aware of only one case of hepatic endometriosis reported in the literature.[2] We describe another patient with a hepatic endometrioma.

Case report

A 37-year-old nulligravid woman who had never undergone laparotomy was referred to us because of chronic, acyclic epigastric pain of a year's duration not accompanied by other symptoms. At abdominal palpation an epigastric mass was identified of hard elastic consistency and smooth surface, moderately tender, and of probable hepatic origin. Results of the abdominal examination were otherwise normal. Liver ultrasonography showed a septate cystic formation of 10 cm in the left lobe, with low-level echoes. An abdominal computed tomography scan revealed a multilocular cyst of 10 cm diameter with fine calcifications in the wall (Fig. 1). Laboratory values were normal; hepatitis B surface antigen and antibodies were negative. A tentative diagnosis was made of *Echinococcus* cyst containing daughter cyst, and surgical exploration was programmed.

At laparotomy with double subcostal incision extended to the xiphoid process, the left liver lobe had a hard elastic mass on the parenchymal surface, connected to the diaphragm by adhesions. Intraoperative ultrasonography after section of the adhesions and the falciform ligament showed that only the second and third hepatic subsegments were involved. A left lateral

From the Departments of Surgery[a] and Obstetrics and Gynecology,[b] University of Milano School of Medicine.
Received for publication April 30, 1990; accepted June 22, 1990.
Reprint requests: Paolo Vercellini, MD, First Department of Obstetrics and Gynecology "L. Mangiagalli," Via Commenda, 12, Milano, Italy 20122.
6/1/23357

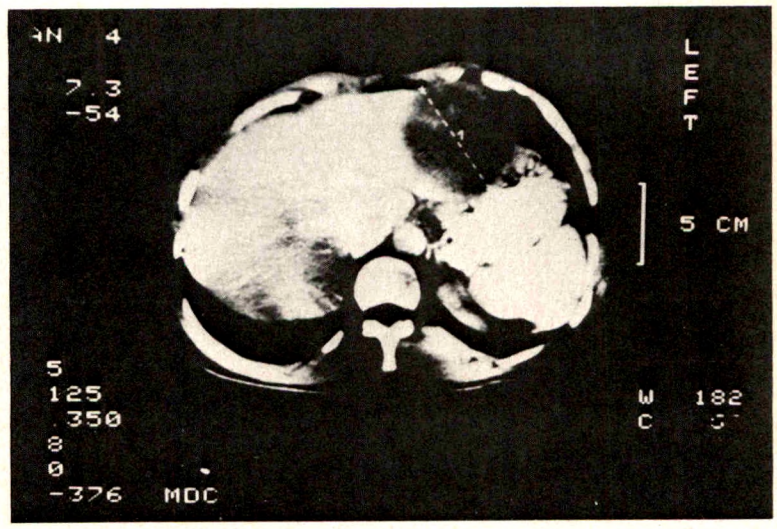


Fig. 1. Computed tomographic scan shows hypodense formation, delimited by wall of greater density containing fine calcifications, located at level of second and third hepatic subsegments.

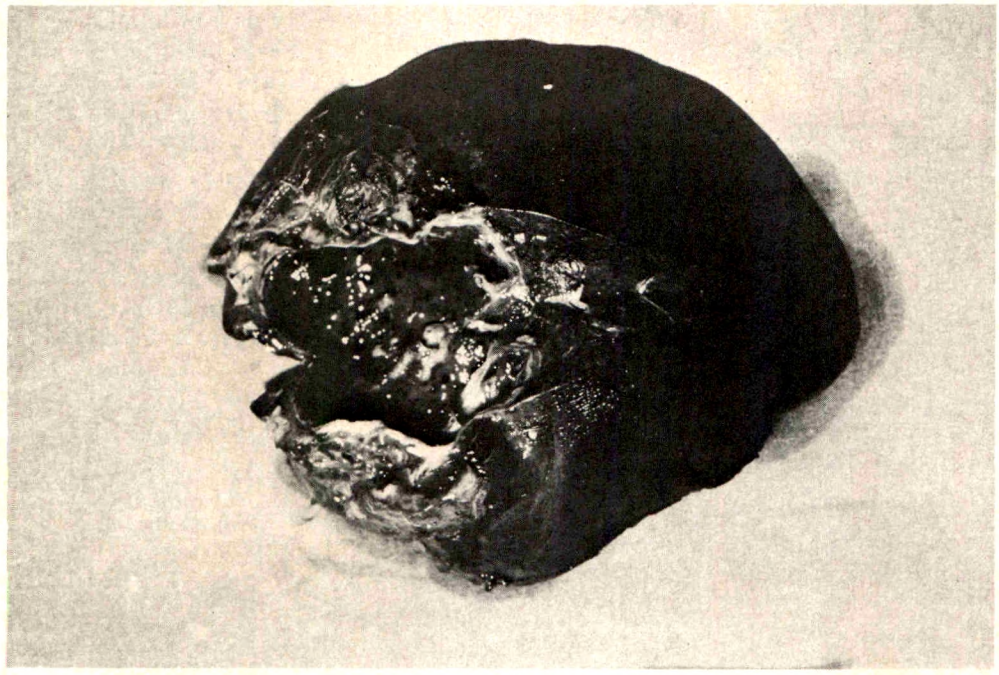


Fig. 2. Left liver segment opened to show endometriotic cyst with typical chocolate-colored material.

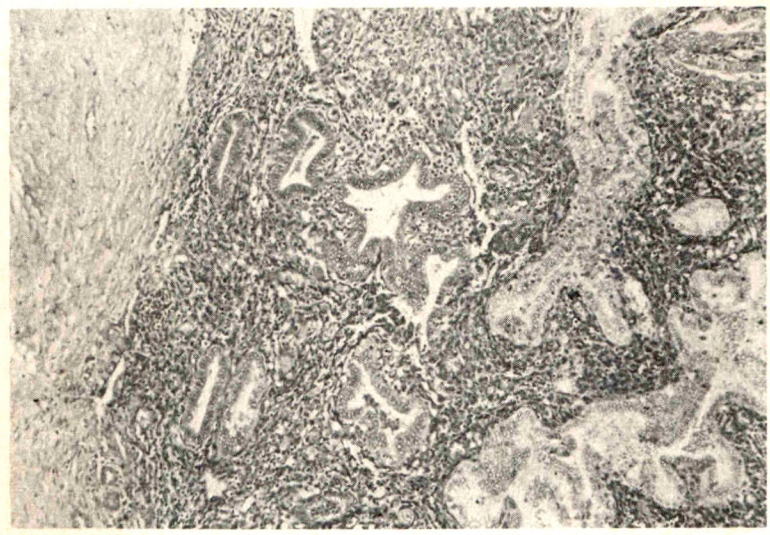


Fig. 3. Histologic preparation of hepatic cyst composed of fibrous wall and internal epithelial lining with endometrial glands and stroma in secretory phase. (Hematoxylin and eosin stain; original magnification ×28.8.)

segmentectomy was performed with removal of the entire cyst (Fig. 2). Gross examination showed a multilocular formation of endometriotic type with the typical dense chocolate-colored content. At histologic examination the cyst wall was lined by endometrial glands and stroma in secretory phase (Fig. 3).

In view of the histologic diagnosis, a gynecologic consultation was requested. The gynecologic history was negative. Pelvic exploration showed a nontender cystic mass at the left adnexal site, confirmed by ultrasonography. The patient underwent a second laparotomy and left adnexectomy was performed because of an ovarian endometrioma of 5 cm diameter. Small peritoneal endometriotic lesions were electrocoagulated.

Danazol 600 mg/day was prescribed postoperatively for 6 months. At 26 months of follow-up the patient was asymptomatic and neither physical examination

nor repeat pelvic and abdominal ultrasonography showed signs of disease recurrence.

Comment

The pathogenesis of a hepatic endometrioma is difficult to explain. The coexistence of pelvic endometriosis in our patient could suggest dissemination through the clockwise intraabdominal circulation of peritoneal fluid. On the other hand, endometrial cells are normally present in the physiologic retrograde menstruation, and even in cases of pelvic endometriosis upper abdominal lesions are extremely rare. In the embryo the paramesonephric ducts derive from an invagination of coelomic epithelium. Furthermore, this epithelium covers the hepatic "bud." Hepatic endometriosis could originate from a spread of müllerian cells in the cranial direction or possibly from coelomic metaplasia of the peritoneum covering the liver. The rarity of the lesion could be related to the distance from the internal genital system.[1]

According to the classification by Markham et al.,[1] our patient had class I endometriosis (involving the intestinal tract), stage I.2.c [no organ defect, intrinsic (parenchyma), >4 cm lesion]. We would not place her in class 0 because this refers to endometriosis that involves sites outside the abdominal cavity.

In view of the absence of pelvic symptoms in our patient and the extreme rarity of hepatic cyst of endometriotic origin, she did not undergo a preoperative gynecologic evaluation. The only other case of hepatic endometrioma reported in the literature of which we are aware presented no indications of associated genital lesions.[2] We believe that endometriosis should be included in the differential diagnosis of a hepatic cyst of unknown cause and that a gynecologic examination and pelvic ultrasonography should be performed before surgery is attempted.

REFERENCES

1. Markham SM, Carpenter SE, Rock JA. Extrapelvic endometriosis. Obstet Gynecol Clin North Am 1989;16:193-219.
2. Finkel L, Marchevsky A, Cohen B. Endometrial cyst of the liver. Am J Gastroenterol 1986;81:576-78.

Antinuclear antibodies in sera of patients with recurrent pregnancy wastage

Ling Xu, MD, Victoria Chang, BS, Ana Murphy, MD, John A. Rock, MD, Marian Damewood, MD, William Schlaff, MD, and Howard A. Zacur, MD, PhD
Baltimore, Maryland

Four groups of women were studied to determine whether low-level antinuclear antibody titers are associated with a higher risk for pregnancy loss. Group A consisted of 30 patients with a history of unexplained fetal losses. Group B consisted of 30 women with "explained" fetal losses (e.g., uterine septum or luteal phase defect). Ages and number of losses were comparable between the women in groups A and B. Group C consisted of 61 healthy pregnant women. Group D involved 61 healthy nonpregnant women of reproductive age. In groups A and B, 40% and 53.3% of the respective patients had antinuclear antibody titers ≥1 : 40. In groups C and D the frequencies of positive antinuclear antibody titers were 8.2% and 5.6%, respectively. This study demonstrates a high prevalence of low-titer antinuclear antibody–positive serum in patients with explained and unexplained pregnancy losses. (AM J OBSTET GYNECOL 1990;163:1493-7.)

Key words: Repeated pregnancy wastage or losses, habitual abortion, antinuclear antibody

Recurrent fetal wastage is common in women with systemic lupus erythematosus,[1-3] presenting even before the onset of clinical manifestations.[4, 5] Since antinuclear antibodies have been accepted as serologic markers for the presence of autoimmune disease,[6-8] the association between recurrent fetal wastage and serum antinuclear antibodies, especially in women who are asymptomatic of any autoimmune disease, has been investigated. No clear relationship has been established until recently when Garcia-De La Torre et al.[9] reported a 30% prevalence of antinuclear antibody-positive sera in patients with unexplained habitual abortions that was significantly greater than 6.6% prevalence in their control group with normal pregnancies. Cowchock et al.[10] reported positive antinuclear antibody titers in four of 14 patients with idiopathic habitual abortion but in only one of 16 patients whose repeated abortions had an explanation (i.e., uterine anomalies, chromosome abnormalities, or luteal phase defect). In a recent prospective study Farnam et al.[11] reported that the incidence of positive antinuclear antibody titers in pregnant women is significantly higher than in nonpregnant women and reaches its highest level at the end of pregnancy.

To determine whether positive antinuclear antibody titers are associated with a higher risk of recurrent fetal wastage or a physiologic change in normal pregnancy, we conducted a study to compare the prevalance of antinuclear antibody titers among patients with unexplained recurrent fetal wastage, patients with explained recurrent fetal wastage, women with normal pregnancy, and healthy nonpregnant women.

Material and methods

Subjects

Patient population. Patients in this study were referred to The Johns Hopkins Hospital from August 1984 to November 1987 for evaluation after at least two *consecutive* pregnancy losses. Clinical and laboratory evaluation in all patients included antinuclear antibody measurement, karyotypic analysis, hysteroscopy or hysterosalpingography, endometrial biopsy or progesterone determinations, and cervical cultures. Thyroid function studies, activated partial thromboplastin time, anticardiolipin panel, mixed lymphocyte culture, or human leukocyte antigen typing were not performed in all patients.

Thirty patients (group A) were identified as having unexplained recurrent fetal wastage because clinical and laboratory evaluation failed to reveal any cause for their pregnancy losses. The mean age of the group was 32.6 years (range, 21 to 38 years). The mean number

From the Division of Reproductive Endocrinology, Department of Gynecology and Obstetrics, The Johns Hopkins University School of Medicine.
Supported in part by National Institutes of Health grant Nos. N01-HD-32816 and 5P30HD06268-16.
Presented as a poster abstract at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Received for publication January 24, 1990; revised June 4, 1990; accepted July 16, 1990.
Reprint requests: Howard A. Zacur, MD, PhD, Park B2-202A, The Johns Hopkins Hospital, 600 North Wolfe St., Baltimore, MD 21205.
6/1/23820

Table I. Possible causes of pregnancy losses in patients with repeated pregnancy losses

Causes	*No.*
Uterine abnormality	16
Luteal phase defect	6
Chromosome abnormality	1
Positive cervical culture	1
Hyperprolactinemia	1
Adrenal hyperplasia	1
Two or more causes combined	4
TOTAL	30

of pregnancy losses was 3.3 (range, 2 to 14). No patient had a history of autoimmune disease.

Another 30 patients (group B) were identified as having explained repeated pregnancy losses because their clinical and laboratory evaluation showed one or more of the following diagnoses: uterine abnormalities, chromosome abnormalities, luteal phase defects, positive cervical culture, or other endocrine and metabolic disorders (Table I). The mean age of this group was 30.4 years (range, 24 to 38 years), and the mean number of pregnancy losses was 2.8 (range, 2 to 6). None had a history of known autoimmune disease.

Normal pregnant population. Serum samples from 61 healthy pregnant women were obtained during routine prenatal visits to the obstetric clinic at The Johns Hopkins Hospital (group C). The mean age of this group was 23.2 years (range, 14 to 35 years). Eighteen women were in their first or second trimester, and 43 were in the third trimester of pregnancy when studied. Individuals with a known history of autoimmune disease were not included in this group.

Healthy nonpregnant population (control group). Sixty-one healthy nonpregnant women of reproductive age (15 to 40 years) were studied (group D). They were considered healthy because none of them had a history of known autoimmune diseases, metabolic disorders, or any chronic or current infectious diseases. These patients had been recruited for another clinical study of oral contraceptive therapy that had been approved by The Johns Hopkins Hospital Joint Committee on Clinical Investigation. None of these patients were taking oral contraceptives at the time of baseline blood sampling.

Antinuclear antibody determination. An indirect fluorescent antibody technique using HEp-2 cell culture and reagents supplied by Zeus Scientific, Inc. (Raritan, N.J.) was used for the antinuclear antibody screening. Sera with 1+ or greater fluorescence at a dilution of 1 : 40 were considered positive.

Statistical analysis. Statistical analysis was performed with the use of t tests for differences between means, and the use of χ^2 statistic with correction for continuity. Significance was determined at a probability level of 0.05 for two-tailed test.

Results

Table II demonstrates the frequency of antinuclear antibody–positive sera in each study group. The frequency of positive antinuclear antibody titers in sera of 30 patients with unexplained repeated pregnancy losses was 40% in comparison with only 5% in 61 healthy nonpregnant women. This difference was statistically significant ($\chi^2 = 15.19$, df = 1, $p < 0.01$). The frequency of antinuclear antibody–positive sera in 30 patients with explained repeated pregnancy losses was 53%; this incidence was also significantly different from the control group ($\chi^2 = 25.42$, df = 1, $p < 0.01$). No significant difference in the frequency of antinuclear antibody–positive sera between patients with explained or unexplained pregnancy losses was found. The mean age and mean frequency of pregnancy losses were also compared between antinuclear antibody–positive patients and antinuclear antibody–negative patients in both explained and unexplained recurrent fetal wastage groups. No significant differences were found (Table III).

In the group of 61 healthy pregnant women (group C) the frequency of antinuclear antibody–positive sera was 8%. Table II shows that there was no significant difference in the frequency of antinuclear antibody–positive sera between normal pregnant women and healthy nonpregnant women (group D) ($\chi^2 = 0.4$, df = 1, $p > 0.05$). Gestational age did not correlate with antinuclear antibody–positive sera (Table IV). Comparison of antinuclear antibody–positive sera between nonpregnant women, pregnant control women, women with unexplained repeated pregnancy losses, and explained pregnancy losses showed no effect of age (Tables III and V).

Comment

It has been recognized that women with autoimmune disease have an increased frequency of pregnancy losses. The recognition of this association has led to general recommendations to screen patients with recurrent fetal wastage for autoimmunity. Reports on the association between recurrent fetal wastage and positive antinuclear antibody titers from several recent studies are controversial. In this study four population groups were examined for the frequency of antinuclear antibody–positive sera, and the differences among these groups in terms of antinuclear antibody–positive sera were compared.

The question of whether recurrent fetal wastage is associated with certain autoimmune disorders has been investigated. Garcia-De La Torre et al.[9] reported that the prevalence of antinuclear antibodies was signifi-

Table II. Frequency of antinuclear antibody–positive sera in each study group

Groups	No.	Antinuclear antibody–positive*		Significance test
		n	%	
Normal control	61	3	5.6	—
Normal pregnancy	61	5	8.2	$p > 0.05$ ($\chi^2 = 0.14$)
Explained recurrent fetal wastage	30	16	53.3	$p < 0.01$ ($\chi^2 = 25.42$)
Unexplained recurrent fetal wastage	30	12	40	$p < 0.01$ ($\chi^2 = 15.80$)

*Positive antinuclear antibody sera defined as having a titer of ≥1:40.

Table III. Comparison of selected variables between antinuclear antibody–negative and antinuclear antibody–positive patients with explained or unexplained recurrent fetal wastage

Variables	Explained recurrent fetal wastage			Unexplained recurrent fetal wastage		
	Antinuclear antibody–negative	Antinuclear antibody–positive	p	Antinuclear antibody–negative	Antinuclear antibody–positive	p
Age (yr) (mean ± SD)	29.4 ± 9.0	29.9 ± 4.0	>0.80	32.6 ± 3.1	31.6 ± 4.7	>0.80
No. of pregnancy losses (mean ± SD)	3.1 ± 1.0	2.5 ± 0.6	>0.05	3.4 ± 2.8	3.3 ± 1.1	>0.80
Frequency of primary recurrent fetal wastage (%)	85.7	93.6	>0.40	72.2	83.3	>0.50

Positive antinuclear antibody sera defined as having a titer of ≥1:40.

Table IV. Association between serum-positive antinuclear antibody and gestational age in normal pregnancy

Gestational age (wk)	No.	Antinuclear antibody–positive		p
		n	%	
<28	18	1	5.60	>0.03
>28	43	4	9.30	
TOTAL	61	5	8.20	

cantly greater in patients with unexplained habitual abortion than in women with normal pregnancies. In a recent controlled prospective study, Petri et al.[12] found that patients with idiopathic habitual abortion did not differ from normal control subjects in the frequency of positive titers for antinuclear antibodies or anti-double-stranded deoxyribonucleic acid. In our investigation antinuclear antibody–positive sera were found in 40% of 30 patients with unexplained recurrent fetal wastage and in 53% of 30 patients with explained pregnancy losses. In comparison, only three (5%) of 61 nonpregnant women and five (8%) of 61 pregnant women had positive antinuclear antibody titers. The differences are statistically significant not only between patients with recurrent fetal wastage and nonpregnant control women, but also between women with recurrent fetal wastage and normal pregnant women. Age did not affect these results. Therefore a positive correlation between pregnancy losses and frequency of antinuclear antibody–positive sera was revealed.

A comparison between patients with unexplained recurrent fetal wastage and patients with explained recurrent fetal wastage was performed. These women were similar in age and number of pregnancy losses. The frequency of antinuclear antibody–positive sera was 40% in cases of unexplained recurrent fetal wastage and 53% in women with explained recurrent fetal wastage. The difference was not significant ($p > 0.05$). Cowchock et al.[10] also reported no difference in rates of antinuclear antibody–positive sera in patients with unexplained recurrent fetal wastage compared with patients with explained recurrent fetal wastage. Because

Table V. Relative odds and age-adjusted relative odds for antinuclear antibody–positive sera

Group	*Odds ratio*	*95% Confidence interval*	*Age-adjusted odds ratio*	*95% Confidence interval*
Normal control	1			
Normal pregnancy	2.7	0.5-14.8	2.0	0.3-13.3
Unexplained recurrent fetal wastage	10.0	2.0-50.1	10.3	1.6-63.6
Explained recurrent fetal wastage	22.7	4.6-112.2	26.6	4.9-145.8

the cause of fetal wastage is complex and undoubtedly multifactorial, other autoimmune disorders could be responsible for repeated pregnancy losses; this may explain the difference between our results and those of other investigators.

We attempted to determine if the presence of serum-positive antinuclear antibodies is a physiologic change of normal pregnancy. Most investigators have found that pregnancy is one factor that may cause exacerbation of systemic lupus erythematosus. Many autoimmune diseases such as systemic lupus erythematosus, progressive systemic sclerosis, autoimmune hemolytic anemia, and autoimmune thrombocytopenia are exacerbated during pregnancy. The association between pregnancy and serum antinuclear antibody has been reported from several studies. Farnam et al.[11] found that antinuclear antibody was detected in 23 of 214 healthy pregnancy women (10.7%), but in only one nonpregnant woman of 50 was antinuclear antibody sera positive (2%). Polishuk et al.[13] reported that serum-positive antinuclear antibody was present in 52.2% of normal pregnant women in comparison with 9.1% of controls. Our investigation revealed no significant difference in the frequency of antinuclear antibody positivity between normal pregnant women (8%) and healthy nonpregnant women (5%), $p > 0.05$; and this concurs with other recent reports.[14,15] Unlike the finding in the study by Farnam et al.,[11] we failed to find an increasing frequency of antinuclear antibody positivity in serum taken from pregnant women later in pregnancy.

Finally, we examined whether other factors were associated with antinuclear antibody–positive sera in patients with recurrent fetal wastage. Cowchock et al.[16] reported that an increased frequency of antinuclear antibody–positive sera was not found in patients with two or more pregnancy losses. We are in agreement with these findings because in our study mean age, order or number of pregnancy losses, and number of previous induced abortions did not affect the frequency of antinuclear antibody–positive sera in women with unexplained or explained recurrent fetal wastage. Our results suggest that a screening test for antinuclear antibodies in patients with recurrent fetal wastage should be considered. Further observations on both the pregnancy outcome and medical course of patients with positive screening studies will be necessary since the clinical significance of the positive antinuclear antibody titer remains to be determined. It also remains unclear whether the positive antinuclear antibody titer preceded or followed the pregnancy losses.

We thank Mrs. Kathy Zulty for her excellent editorial assistance.

REFERENCES

1. Junco DJ, et al. Association of autoimmune condition with recurrent intrauterine death. Clin Obstet Gynecol 1986; 29:959.
2. Mowbray JF, Underwood JL. Immunology of abortion. Clin Exp Immunol 1985;60:1.
3. Varner MW, Meehan RT, Syrop CH, Strottmann MP, Goplerud CP. Pregnancy in patients with systemic lupus erythematosus. AAM J OBSTET GYNECOL 1985;145:1025-37.
4. Gardlund B. The lupus inhibitor in thromboembolic disease and intrauterine death in the absence of systemic lupus. Acta Med Scand 1984;215:293-8.
5. Fraga A, Mintz G, Orozco J, and Orozco JH. Sterility and fertility rates, fetal wastage and maternal morbidity in systemic lupus erythematosus. J Rheumatol 1974;1: 293-8.
6. Ferrel PB, Tan EM. Systemic lupus erythematosus. In: Rose NR, Mackay IR, eds. The autoimmune diseases. Orlando: Academic Press, 1985:29.
7. Davis JS. Antinuclear antibodies (ANA). In: Kelley WN, Harris ED, Ruddy S, Sledge CB, eds. Textbook of rheumatology. Philadelphia: WB Saunders, 1981 vol 1:691-709.
8. Syrop CH, Varner MW. Systemic lupus erythematosus. Clin Obstet Gynecol 1983;26:547.
9. Garcia-De La Torre I, Hernandez-Vazquez L, Angulo-Vazquez J, Romero-Ornelas A. Prevalence of antinuclear antibodies in patients with habitual abortion and in normal and toxemic pregnancies. Rheumatol Int 1984;4:87.
10. Cowchock S, Dehoratius RD, Wapmer RJ, Jackson LG. Subclinical autoimmune disease and unexplained abortion. AM J OBSTET GYNECOL 1984;150:367-71.
11. Farnam J, Lavastida MT, Grant JA, Reddi RC, Daniels JC. Antinuclear antibodies in the serum of normal pregnant women: a prospective study. J Allerg Clin Immunol 1984;73:596-99.
12. Petri M, Golbus M, Anderson R, Whiting-O'Keefe Q, Corash L, Hellman D. Antinuclear antibody, lupus anticoagulant, and anticardiolipin antibody in women with idiopathic habitual abortion: a controlled, prospective study of forty-four women. Arthritis Rheum 1987;30:601-6.

13. Polishuk WZ, Beyth Y, Izak G. Antinuclear factor and LE cells in pregnant women [Letter]. Lancet 1971;2:270.
14. Hess EV, Baum J. Antinuclear factor and LE cells in pregnant women. Lancet 1971;2:871.
15. Hinkle SC, Merkatz IR, Gyves MT, Michel B, Miskowitz RW. Antinuclear factor and anti-DNA in sera of pregnant women [Letter]. Arthritis Rheum 1979;22:201.
16. Cowchock S, Smith JB, Gocial B. Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. AM J OBSTET GYNECOL 1986;155:1002-10.

Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels

Paul G. Kratzer, MD, PhD, and Robert N. Taylor, MD, PhD
San Francisco, California

Regulation of human corpus luteum activity was studied with radioimmunoassays and bioassays of sera drawn serially from women suspected of having ectopic pregnancies. Progesterone values exhibited considerable overlap in pregnancies that were subsequently classified as normal intrauterine (n = 21), ectopic (n = 35), or spontaneous abortion (n = 14). The rate of change of human chorionic gonadotropin concentration was significantly correlated with progesterone levels in ectopic (r = 0.64) and all pregnancies (r = 0.70). There was no correlation (r = −0.18) between the rate of change of human chorionic gonadotropin and the bioactivity produced per volume of serum. Ectopic pregnancies and normal intrauterine pregnancies did not differ in their ratio of in vivo bioactivity to immunoactivity. From these data we conclude that corpus luteum activity is primarily regulated by the rate of change of human chorionic gonadotropin concentration, without the involvement of other serum factors. We also conclude that reduced corpus luteum function in ectopic pregnancies is not a result of biochemical modification of the human chorionic gonadotropin molecule. Finally, we discourage the use of single progesterone values in screening for ectopic pregnancies because of the considerable overlap in progesterone values among these and normal intrauterine pregnancies. (AM J OBSTET GYNECOL 1990;163:1497-502.)

Key words: Corpus luteum, ectopic pregnancy, chorionic gonadotropin, progesterone

The human corpus luteum is formed from the remnants of the postovulatory ovarian follicle. An important function of the corpus luteum is the synthesis of progesterone. Progesterone synthesized from the corpus luteum is required for the maintenance of normal pregnancy for the first 7 weeks' gestation.[1]

From the Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco.
Supported by a grant from the Obstetrics and Gynecology Research and Education Foundation and by the Radioimmunoassay Core of HD 11979.
Received for publication March 6, 1990; revised June 4, 1990; accepted July 27, 1990.
Reprint requests: Robert N. Taylor, MD, PhD, W. M. Keck Laboratory of Reproductive and Developmental Molecular Biology, Department of Obstetrics, Gynecology and Reproductive Sciences, Room M-1489, University of California, San Francisco, CA 94143-0132.
6/1/24144

The placental syncytiotrophoblast synthesizes increasing amounts of progesterone, such that by 8 1/2 weeks the placenta and corpus luteum contribute equal amounts of progesterone and the placental contribution alone is sufficient to maintain pregnancy.[1] Overall, the level of progesterone normally increases for the first 2 weeks after conception while luteinization is prominent, and then slightly decreases or remains approximately constant until the luteoplacental shift.[2-4]

The regulation of the corpus luteum is poorly understood. Human chorionic gonadotropin (hCG) administered to nonpregnant women in the luteal phase prolongs the life of the corpus luteum[5-8] and enhances the production of progesterone.[9] However, a simple dose-response relationship does not exist for hCG as evidenced by the relatively constant progesterone levels in early normal intrauterine pregnancies in which the

hCG levels vary remarkably. Abnormalities of early human pregnancy and placentation, such as ectopic pregnancy and spontaneous abortion, provide opportunities to evaluate corpus luteum pathophysiology.

In ectopic pregnancies the progesterone levels are generally lower than in normal intrauterine pregnancies.[10,11] However, these lower progesterone levels are not a result of the lower absolute hCG levels that are found in ectopic pregnancies, inasmuch as when ectopic pregnancies and intrauterine pregnancies with similar hCG levels were compared, the progesterone levels in ectopic pregnancies were still lower.[12] The cause of the lower progesterone levels in ectopic pregnancies could be explained by several possible mechanisms: (1) ectopic pregnancies might synthesize an inferior quality hCG, perhaps as a result of aberrant glycosylation, thereby rendering it less efficient in the stimulation of corpus luteum production of progesterone; (2) a specific factor(s) other than hCG might be responsible for corpus luteum stimulation and this factor(s) could be deficient in ectopic pregnancies; (3) corpus luteum activity might be dependent on the rate of hCG production, which is lower in ectopic pregnancies.[13]

As a consequence of the lower progesterone levels noted in ectopic pregnancies, it has been proposed that ectopic pregnancies could be distinguished from normal intrauterine pregnancies on this basis.[12,14-17] We have studied a population of women who were first seen in subacute condition and had serial blood samples drawn as part of an evaluation to rule out ectopic pregnancy. Analysis of these samples allowed us to assess the basis of corpus luteum regulation and to evaluate the reliability of a single progesterone value in screening for ectopic pregnancies.

Material and methods

Patients. Seventy patients were identified retrospectively from 1986 to 1990 who were first seen at either Moffitt Hospital or San Francisco General Hospital within the first 10 weeks' gestation (5.6 ± 0.2 weeks, mean ± SE) in subacute condition with signs and symptoms that were suspicious for ectopic pregnancy. All patients had conceived after spontaneous ovulation and had not received postconceptional hormone support. Part of the management of these patients consisted of serial hCG levels drawn over several days. The sera were subsequently stored at −20° C. The patients were ultimately classified into three groups by subsequent outcome: (1) ectopic pregnancy verified by pathologic examination of the surgical specimen, (2) viable intrauterine pregnancy verified by follow-up prenatal care to at least midgestation, or (3) clinical spontaneous abortion.

Assays. The frozen sera were evaluated with the following assays. The hCG was assayed by a double-antibody radioimmunoassay that detected total β-hCG. The hCG levels were expressed as milliinternational units (mIU) with the Second International Standard for hCG. The intra- and interassay coefficients of variation were 8% and 14%, respectively. Progesterone was assayed after hexane extraction with a standard radioimmunoassay with intra- and interassay coefficients of variation of 9% and 12%, respectively. In vivo bioactivity of hCG was determined with a mouse uterine weight assay.[18] Serum from each patient was serially diluted in saline solution after normalizing to a constant concentration by radioimmunoassay. The patients' sera were then injected intraperitoneally into 4-week-old Balb/c female mice on three successive days. On the fourth day the mice were killed. Each uterus was dissected free of the cervix, oviducts, and mesenteries, incised longitudinally, blotted dry, and then weighed. Uterine weight increased linearly from 10 to 50 mg with hCG concentration over the range of 15 to 100 mIU/ml using Profasi hCG (Serono Laboratories, Randolph, Mass.) as a standard. The bioactivity to immunoactivity ratio of serum hCG from each patient was determined by comparison of the slope of the curve to that of the standard hCG curve. The intra- and interassay coefficients of variation of the bioactivity to immunoactivity ratio were 19% and 21%, respectively. The mouse bioassay was conducted in accordance with the guidelines outlined in the JOURNAL and with permission of the University of California, San Francisco Committee on Animal Research.

Calculation of the rate of change of hCG. The rate of change of hCG was determined from the slope of the curve generated by plotting hCG concentration versus date and time of serial blood samples drawn at 2- to 3-day intervals. The rate of change was expressed as the number of hCG doublings per day, which is the reciprocal of the doubling time. By so expressing the rate of change, a continuum of negative-to-positive changes could be plotted, representing decreasing and increasing hCG concentrations, respectively. This method of expressing the rate of change rather than as the doubling time avoids the discontinuity in the plot that would otherwise occur for the situation when there is no change in hCG concentration.

Statistics. Differences between patient groups were assessed with the two-tailed, unpaired Student t test, with $p < 0.05$ indicating significance.

Results

The rate of change of hCG is expressed as the number of hCG doublings per day, which is the reciprocal of the doubling time. In agreement with previously

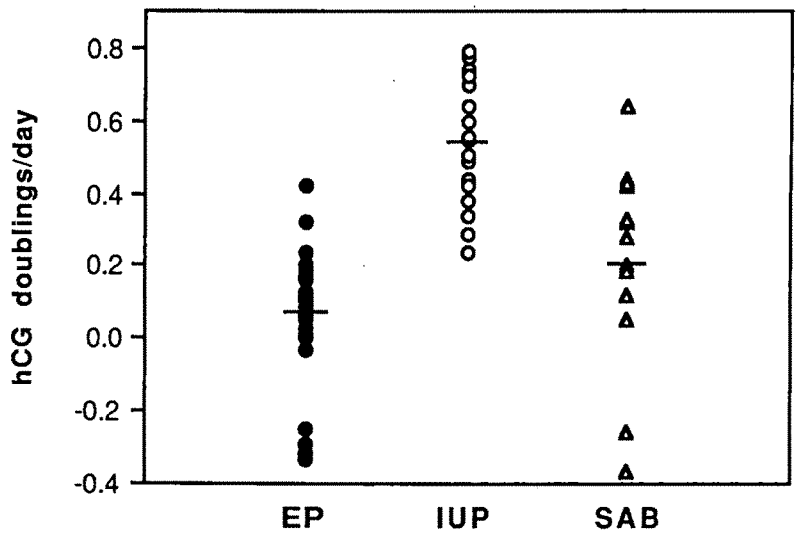


Fig. 1. Number of hCG doublings per day for individual samples from ectopic pregnancies (*EP*, •), normal intrauterine pregnancies (*IUP*, ○), and spontaneous abortions (*SAB*, △). Means for each group are indicated by *horizontal bars*. Mean rate for ectopic pregnancies was significantly less than that for intrauterine pregnancies ($p < 0.05$, by Student t test).

published results, the rate of change of hCG was significantly lower in ectopic pregnancies than that in normal intrauterine pregnancies (0.07 ± 0.03 hCG doublings per day vs 0.56 ± 0.04 hCG doublings per day, $p < 0.05$) with an intermediate rate determined for spontaneous abortions (0.21 ± 0.07 hCG doublings per day). The rates for individual samples are displayed in Fig. 1 and it is seen that samples from intrauterine pregnancies, spontaneous abortions, and ectopic pregnancies have overlapping values. Of ectopic pregnancies, 5/35 had a number of hCG doublings per day that equaled or exceeded the lowest rate observed in a normal intrauterine pregnancy (0.24 hCG doublings per day). Half the spontaneous abortions also had rates approximating those of the normal pregnancies.

Mean progesterone values were also significantly lower in ectopic pregnancies than in intrauterine pregnancies (10.5 ± 1.2 vs 29.2 ± 2.4 ng/ml, $p < 0.05$) and again were intermediate in spontaneous abortions (17.2 ± 3.1 ng/ml). It is apparent from Fig. 2 that there was considerable overlap among individual samples from intrauterine pregnancies, spontaneous abortions, and ectopic pregnancies. With various progesterone cutoff levels, 9/35 ectopic pregnancies had progesterone levels >15 ng/ml, a level that all normal intrauterine pregnancies exceeded, whereas 6/35 ectopic pregnancies had progesterone levels >20 ng/ml compared with 16/21 normal intrauterine pregnancies. For pregnancies resulting in spontaneous abortions, 8/14 had progesterone levels <20 ng/ml and 5/14 had progesterone levels <15 ng/ml.

The progesterone levels for individual patients were correlated significantly with an increasing number of hCG doublings per day in ectopic pregnancies ($r = 0.64$) and for all pregnancies ($r = 0.70$) as shown in Fig. 3. In contrast, there was no correlation between progesterone levels for individual patients and the absolute level of hCG for all pregnancies ($r = 0.02$). If we consider only those pregnancies in which serum samples were obtained at ≤7 weeks' gestation (i.e., before the luteoplacental transition begins), the correlation between progesterone levels and hCG doublings remains high for ectopic pregnancies ($r = 0.78$, $n = 20$) and for all pregnancies ($r = 0.67$, $n = 52$). Thus our findings do not appear to be skewed by placental steroidogenesis.

The mean bioactivity to immunoactivity ratio was not different among 12 ectopic pregnancies and 12 normal intrauterine pregnancies (9.2 ± 0.8 vs 7.7 ± 1.3, $p > 0.1$) (Table I). Likewise, when the bioactivity was considered per volume of serum, there was no correlation with the rate of change of hCG for either ectopic pregnancies alone ($r = -0.17$) or for ectopic pregnancies and intrauterine pregnancies ($r = -0.18$).

Comment

Corpus luteum function is required to maintain the early pregnancy until the luteoplacental shift begins at approximately 7 weeks' gestation.[1] Administration of hCG to nonpregnant women during the luteal phase stimulates corpus luteum activity[5-8] and prolongs its life span.[9] The role of hCG is unclear because there is no simple dose-response relationship. Possibilities include a requirement for the presence of an appropriate rate

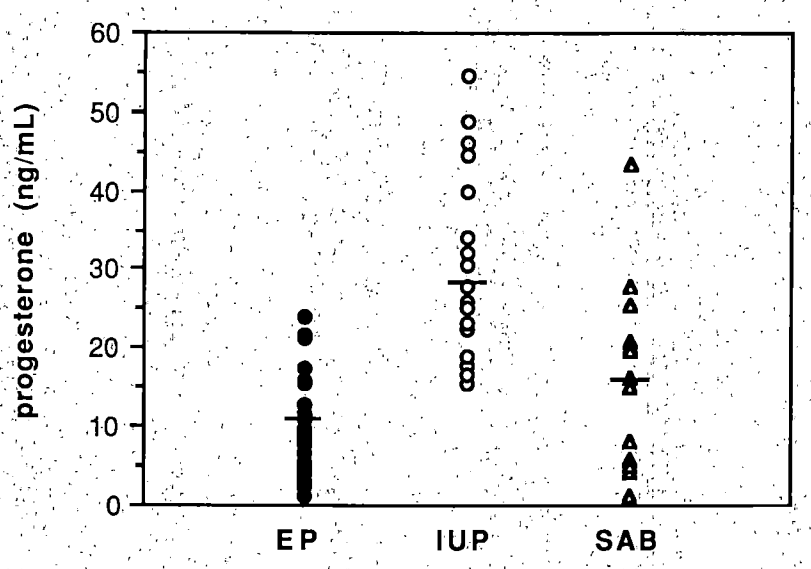


Fig. 2. Progesterone values (ng/ml) for individual samples from ectopic pregnancies (*EP*, •), normal intrauterine pregnancies (*IUP*, ○), and spontaneous abortions (*SAB*, △). Means for each group are indicated by *horizontal bars*. Mean progesterone value for ectopic pregnancies was significantly less than that for intrauterine pregnancies ($p < 0.05$, by Student t test).

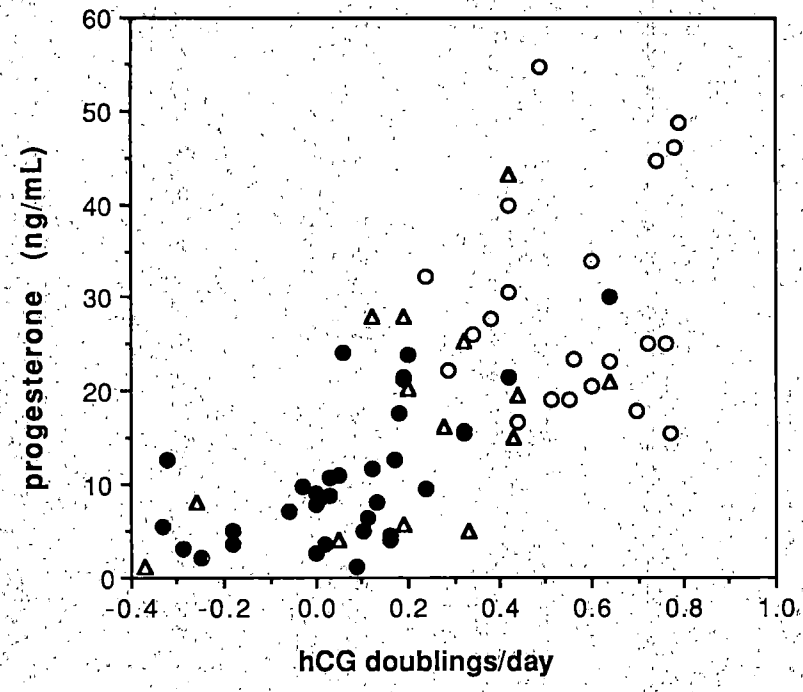


Fig. 3. Number of hCG doublings per day plotted against progesterone values (ng/ml) for individual samples from ectopic pregnancies (•), normal intrauterine pregnancies (○), and spontaneous abortions (△). Positive correlations were observed when all samples were considered ($r = 0.70$) and when ectopic pregnancies were considered separately ($r = 0.64$).

of change of hCG or another factor besides hCG that correlates with hCG. In this article, which expands on our preliminary report (Kratzer and Taylor, Proceedings of the forty-fifth meeting of the American Fertility Society, 1989:102), we show that corpus luteum activity is significantly correlated with the rate of change of hCG. The correlation of progesterone level with the rate of change of hCG obtains for all pregnancies in our study and most importantly it obtains in pregnancies before the luteoplacental shift when progesterone

levels overwhelmingly reflect the activity of the corpus luteum.[1] This correlation is not surprising in light of the lower progesterone levels in ectopic pregnancies[10, 11] and the lower rate of change of hCG in ectopic pregnancies[13] compared with normal intrauterine pregnancies. The possibility that another factor is involved in corpus luteum regulation can be excluded to a large extent by the following argument. If another factor were important in corpus luteum regulation, then it must be correlated with the rate of change of hCG, so that sera from patients with more rapidly increasing hCG should produce a greater response per volume of serum in the bioassay. However, no such correlation exists. Our bioassay was a heterologous system that used the mouse uterus. It is conceivable, albeit unlikely, that a factor other than hCG might exist that is functional in human beings, but not in the mouse. Except for this caveat, corpus luteum function is primarily regulated by the rate of change of hCG production.

Dependency of corpus luteum activity on the rate of change of hCG, rather than on its absolute concentration, explains why normal early intrauterine pregnancies of different ages and widely different hCG concentrations, but all with rapidly increasing hCG levels, have similar progesterone levels. The dependency of corpus luteum activity on the rate of change of hCG also explains why ectopic pregnancies and spontaneous abortions often have lower progesterone levels than normal intrauterine pregnancies, as the former generally have less rapidly increasing hCG levels. Dependency on the rate of change of hCG also explains why there is only limited prolongation of corpus luteum life span in nonpregnant women after chronic administration of a constant dose of hCG.[9] In the monkey, chronic administration of a constant dose of hCG also results in only limited prolongation of the corpus luteum.[19] However, it has been found that corpus luteum life span can be extensively prolonged by continuously increasing the hCG dose in the early luteal phase.[20]

The mechanism by which the rate of hCG presentation regulates corpus luteum function may involve the number and percentage of bound luteinizing hormone/hCG receptors on the corpus luteum. In early human pregnancy, the number of unbound luteinizing hormone/hCG receptors is low and increases in the first trimester.[21] This low number of unbound receptors is a result of a high receptor occupancy rate[22, 23] rather than a result of down regulation in the total number of receptors.[23] In persistently stimulated monkey corpus luteum likewise, both the number of occupied receptors and total number of receptors increases, whereas the number of unoccupied sites remains unchanged.[24] We propose that in the first 2 weeks' gestation, the percentage of ligand-bound receptors on the corpus luteum increases from a very small percentage to a percentage at which maximal stimulation is achieved and that the number of these receptors increases as a result of corpus luteum growth and maturation. An increasing number of luteinizing hormone/hCG receptors on the corpus luteum cells would require that hCG concentrations increase at a sufficient rate to maintain optimal receptor occupancy. By this mechanism, maximal corpus luteum stimulation would be achieved only when hCG production increased commensurately with the number of receptors.

Table I. Bioactivity/immunoactivity ratio in ectopic pregnancies and normal intrauterine pregnancies

Sample	*Number*	*Ratio**
Ectopic pregnancy	12	9.2 ± 0.8
Normal intrauterine pregnancy	12	7.7 ± 1.3

*Bioactivity/immunoactivity ratio values are expressed as the mean ± SE. The difference between the means was not significant ($p > 0.1$, by the Student t test).

In ectopic pregnancies, the lower progesterone levels could result solely from a decreased rate of change of hCG, or could be partly a result of decreased biopotency of hCG or the lack of another factor. As argued previously, it is doubtful that there is another factor that is lacking. Reduced biopotency of the hCG molecule in ectopic pregnancies compared with that in normal intrauterine pregnancies has not been found in either heterologous (mouse Leydig cell) in vitro bioassays,[25, 26] or in homologous (human granulosa cell) in vitro bioassays (Kratzer, Rabinovici, and Taylor, unpublished results). The absence of a difference in these in vitro bioassays suggested that altered glycosylation of hCG does not explain the impaired corpus luteum function in ectopic pregnancies. Our in vivo bioassay could have detected additional modifications of hCG molecules, such as sialylation, that would affect its half-life. The lack of a difference in hCG bioactivity between ectopic pregnancies and intrauterine pregnancies in our in vivo bioassay therefore mitigates against post-translational modifications of hCG in ectopic pregnancies such as glycosylation and sialylation. Therefore in ectopic pregnancies impaired hCG production by the abnormally implanted trophoblast appears to be primarily responsible for the inability of the corpus luteum to maintain optimal function.

Ectopic pregnancies are increasing in frequency and represent a potential threat to the mother's life and future fertility.[27] Classically, surgery has been used for the diagnosis and treatment of ectopic pregnancies first seen in an acute condition, whereas protocols that involve serial hCG determinations and ultrasonographic evaluation have been used in the diagnosis of those first

seen in a subacute condition. Recently, the use of single progesterone values has been advocated to screen for ectopic pregnancies with progesterone cutoffs of 20 ng/ml,[16] 15 ng/ml,[12, 15] 10 ng/ml,[14] or 6.5 ng/ml.[17] It was further recommended that patients with progesterone levels below these cutoffs should undergo uterine curettage for a presumed abnormal pregnancy, and if no villi were found in the curettings that laparoscopy or methotrexate therapy be instituted.[15,17] On the basis of the findings reported here, we discourage the use of single progesterone levels to screen for ectopic pregnancies because a considerable number of ectopic pregnancies would be missed and these would still need to be followed by serial hCG levels with the current protocols. Moreover, the possibility of inadvertently mistaking an early, normal intrauterine pregnancy with a low progesterone value for an abnormal pregnancy, and consequently terminating such a pregnancy, is disconcerting. To avoid this type of error, the cutoff would have to be set so low that screening with progesterone would identify too few ectopic pregnancies to be of value.

We thank Dr. Scott Monroe and Mary Tono for their generous help with specimen collection and Carmelita Aquirre and Steven Zippin for their excellent technical assistance.

REFERENCES

1. Csapo AI, Pulkkinen M. Indispensability of the human corpus luteum in the maintenance of early pregnancy. Obstet Gynecol Survey 1978;33:69-81.
2. Johansson EDB. Plasma levels of progesterone in pregnancy measured by a rapid competitive protein binding technique. Acta Endocrinol 1969;61:607-17.
3. Tulchinsky D, Hobel CJ. Plasma human chorionic gonadotropin, estrone, estradiol, estriol, progesterone, and 17 α-hydroxyprogesterone in human pregnancy. III. Early normal pregnancy. Am J Obstet Gynecol 1973;117:884-93.
4. Corker CS, Michie E, Hobson B, Parboosingh J. Hormonal patterns in conceptual cycles and early pregnancy. Br J Obstet Gynaecol 1976;83:489-94.
5. Browne JSL, Venning EH. The effect of intramuscular injection of gonadotropic substances on the corpus luteum phase of the human menstrual cycle. Am J Physiol 1938;123:26.
6. Brown WE, Bradbury JT. A study of the physiologic action of human chorionic hormone: the production of pseudopregnancy in women by chorionic hormone. Am J Obstet Gynecol 1947;53:749-57.
7. de Watteville H. Effets des gonadotrophines en doses massives. Gynaecol 1948;126:318-9.
8. Fried PH, Rakoff AE. The effects of chorionic gonadotropin and prolactin on the maintenance of corpus luteum function. J Clin Endocrinol Metab 1952;12:321-37.
9. Strott CA, Yoshimi T, Ross GT, Lipsett MD. Ovarian physiology: relationship between plasma LH and steroidogenesis by the follicle and corpus luteum; effect of hCG. J Clin Endocrinol Metab 1969;29:1157-67.
10. Milwidsky A, Adoni A, Segal S, Palti Z. Chorionic gonadotropin and progesterone levels in ectopic pregnancy. Obstet Gynecol 1977;50:145-7.
11. Radwanska E, Frankenberg J, Allen EI. Plasma progesterone levels in normal and abnormal early human pregnancy. Fertil Steril 1978;30:398-402.
12. Hubinont CJ, Thomas C, Schwers JF. Luteal function in ectopic pregnancy. Am J Obstet Gynecol 1987;156:669-74.
13. Kadar N, Caldwell BV, Romero R. A method of screening for ectopic pregnancy and its indications. Obstet Gynecol 1981;58:162-6.
14. Matthews CP, Coulson PB, Wild RA. Serum progesterone levels as an aid in the diagnosis of ectopic pregnancy. Obstet Gynecol 1986;68:390-4.
15. Yeko TR, Gorrill MJ, Hughes LH, Rodi IA, Buster JE, Sauer MV. Timely diagnosis of early ectopic pregnancy using a single blood progesterone measurement. Fertil Steril 1987;48:1048-50.
16. Buck RH, Joubert SM, Norman RJ. Serum progesterone in the diagnosis of ectopic pregnancy: a valuable diagnostic test? Fertil Steril 1988;50:752-5.
17. Stovall TG, Ling FW, Cope BJ, Buster JE. Preventing ruptured ectopic pregnancy with a single serum progesterone. Am J Obstet Gynecol 1989;160:1425-31.
18. Levin L, Tyndale HH. The quantitative assay of 'follicle-stimulating' substances. Endocrinology 1937;21:619-28.
19. Hisaw FL. The placental gonadotrophin and luteal function in monkeys *(Macaca mulatta)*. Yale J Biol Med 1944;17:119-37.
20. Ottobre JS, Stouffer RL. Persistent versus transient stimulation of the macaque corpus luteum during prolonged exposure to human chorionic gonadotropin: a function of age of the corpus luteum. Endocrinology 1984; 114:2175-82.
21. Rajaniemi HJ, Ronnberg L, Kauppila A, et al. Luteinizing hormone receptors in human ovarian follicles and corpora lutea during menstrual cycle and pregnancy. J Clin Endocrinol Metab 1981;52:307-13.
22. Halme J, Ikonen M, Rutanen EM, Seppala M. Gonadotropin receptors of human corpus luteum during menstrual cycle and pregnancy. Am J Obstet Gynecol 1979;131:728-34.
23. Bramley TA, Stirling D, Swanson IA, Menzies GS, McNeilly AS, Baird DT. Specific binding sites for gonadotropin-releasing hormone, LH/chorionic gonadotrophin, low-density lipoprotein, prolactin and FSH in homogenates of human corpus luteum. II: Concentrations throughout the luteal phase of the menstrual cycle and early pregnancy. J Endocrinol 1987;113:317-27.
24. Vandevoort CA, Stouffer RL, Molskness TA, Ottobre JS. Chronic exposure of the developing corpus luteum in monkeys to chorionic gonadotropin: persistent progesterone production despite desensitization of adenylate cyclase. Endocrinology 1988;122:1876-82.
25. Norman RJ, Buck RH, Kemp MA, Joubert SM. Impaired corpus luteum function in ectopic pregnancy cannot be explained by altered human chorionic gonadotropin. J Clin Endocrinol Metab 1988;66:1166-70.
26. Sauer MV, Anderson RE, Vermesh M, Stone BA, Paulson RJ. Spontaneously resorbing ectopic pregnancy: preservation of human chorionic gonadotropin bioactivity despite declining steroid hormone levels. Am J Obstet Gynecol 1989;161:1673-6.
27. Taylor RN. Ectopic pregnancy and reproductive technology. JAMA 1988;259:1862-4.

Hemoperitoneum as a result of coital injury without associated vaginal injury

Sterling W. McColgin, MD,[a, b] Lisa M. Williams, MD,[b] Timothy L. Sorrells, MD,[a] and John C. Morrison, MD[b]

Travis Air Force Base, California, and Jackson, Mississippi

Hemoperitoneum as a result of coital injury without associated vaginal injury is an extremely rare entity, and evidenced by only five cases that have been reported in the medical literature to date. We report five additional cases encountered in two medical centers. Two of these were ruptured corpus luteum cysts, one was a laceration of the round ligament, another was a laceration of an ovary, and the fifth was rupture of a serous cystadenoma. This diagnosis should be considered in patients with hemoperitoneum after coitus. (Am J Obstet Gynecol 1990;163:1503-5.)

Key words: Hemoperitoneum, coital unjury, culdocentesis

Since 1907, 232 cases of significant female coital injuries were found in the medical literature.[1-15] Of these, there were only five reported cases of hemoperitoneum caused by coital injury without associated vaginal injury. Five additional cases of this most unusual entity were diagnosed and treated by us. All of these additional patients had normal results of vaginal examination.

Case reports

A 31-year-old woman, para 3-0-0-3, had sudden onset of lower abdominal pain during coitus on the evening before admission. The pain had increased and by morning radiated to her back and shoulders. Her last menstrual period was 3 weeks before admission. She had undergone a postpartum tubal ligation with her last cesarean delivery. The serum human chorionic gonadotropin level was normal. Abdominal examination revealed diffuse tenderness with mild bilateral rebound and normal bowel sounds. Pelvic examination revealed no trauma to the vagina; the uterus was of normal size with moderate bilateral adnexal tenderness. Culdocentesis produced 10 ml of nonclotting thin, bloody fluid. The diagnosis of ruptured follicular cyst was made. Vital signs were evaluated frequently, and serial hemoglobin and hematocrit levels were determined. Because of a decline in the hemoglobin from 11.2 to 8.7 gm/dl, an exploratory laparotomy was performed. At exploration, a hemoperitoneum was encountered. The tubes, ovaries, and uterus were grossly normal except for evidence of the previous tubal ligation. No adhesions were noted. Further inspection revealed a 2 to 3 cm laceration of the right round ligament with active arterial bleeding. Hemostasis was obtained with figure-of-eight sutures. After an uneventful postoperative course, she was discharged on the fifth postoperative day.

A 30-year-old woman, para 0-1-4-1, presented to our emergency room with a 5-hour history of abdominal pain that started 3 hours after coitus and gradually worsened. Her last menstrual period was 3 weeks before admission, and she was not using any contraceptive measures. Her history was significant for use of an intrauterine contraceptive device, gonorrhea with subsequent pelvic inflammatory disease, and two previous cesarean sections. On evaluation, the patient was noted to have an acute abdomen and a negative serum pregnancy test result. An exploratory laparotomy was performed with findings of 500 ml of blood intraabdominally and a 2.5 cm laceration on the right ovary. There was no evidence of adhesive disease or pelvic inflammatory residual intraabdominally at the time of operation. The laceration was repaired with interrupted sutures. She was discharged on the fourth postoperative day without complications.

Two hours after coitus, a 36-year-old woman presented to our emergency room with the onset of sudden sharp and increasing abdominal pain radiating to the right shoulder. She had a tubal ligation in 1975 and a hysterectomy and appendectomy in 1981. On evaluation, she had a positive psoas sign and was believed to have an acute abdomen. An exploratory laparotomy was performed with findings of 1000 ml of blood in the abdomen and a 3 cm ruptured ovarian cyst that was adherent to the left pelvic sidewall. A left salpingo-oophorectomy was performed. Numerous bowel and

From the David Grant Medical Center[a] and the Department of Obstetrics and Gynecology, University of Mississippi Medical Center.[b]
Supported in part by the Vicksburg Hospital Medical Foundation.
Opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Air Force or the Department of Defense.
Received for publication January 10, 1990; revised July 6, 1990; accepted July 16, 1990.
Reprint requests: Sterling W. McColgin, MD, Department of Obstetrics and Gynecology, Box B-198, University of Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO 80262.
6/1/23777

pelvic adhesions were noted intraoperatively and were lysed. Her postoperative course was complicated because of chronic obstructive pulmonary disease and postoperative pneumonia. She was discharged on the eleventh postoperative day. Pathologic diagnosis of the ovary revealed a serous cystadenoma that had ruptured.

A 25-year-old woman, para 2-0-0-2, noted the onset of sudden suprapubic pain with radiation to the right lower quadrant and right shoulder during coitus. During evaluation, a bulging cul-de-sac was noted and culdocentesis resulted in 15 ml of nonclotting blood. She was orthostatic at the time of evaluation and immediately given fluid for resuscitation and prepared for operation. She had never been hospitalized except for two spontaneous vaginal deliveries. An exploratory laparotomy was performed and 350 ml of blood and blood clot was found in the abdomen. In addition, a bleeding corpus luteum cyst was noted at the distal pole of the left ovary. A wedge resection was performed and hemostasis was ascertained. The other ovary was normal and there was no evidence of prior pelvic infection, adhesions, or endometriosis. She was discharged on the fourth postoperative day after an uneventful postoperative course.

A 24-year-old, nulliparous patient presented to the emergency room with bilateral lower quadrant pain. During coitus on the evening of admission, the patient noted a dull aching pain deep inside the pelvis and on withdrawal felt a severe pain "like a balloon inside bursting." The pain continued to worsen with localization to the right lower quadrant. Previous surgery included a diagnostic laparoscopy for an infertility evaluation with negative findings. Her history was also positive for severe dysmenorrhea. The patient was admitted and underwent a diagnostic laparoscopy. An active bleeding site was seen on the right ovary, and at subsequent laparotomy a ruptured hemorrhagic corpus luteum cyst was wedged resected. The other adnexa and the uterus were normal, and there was no evidence of previous gynecologic disease. She was discharged on the fifth postoperative day after an uncomplicated postoperative course.

Comment

The pelvic organs are well suited for intercourse and coital injuries are believed to be unusual. Until the end of the nineteenth century, it was doubted that lesions could occur at all during coitus that took place with the common consent of both parties.[8] This premise was disproved by Neugebauer[16] in 1895, who showed that the mere insertion of the penis during intercourse can cause lesions in all parts of the vulva and vagina, including the vault.

Most reviews of coital trauma and discussions of the etiologic factors involved limit their discussions to vaginal and perineal injuries. Reported predisposing factors include (1) pregnancy—with the increased friability of the tissues and fixation of the genital tract coincident with gestation; (2) vigorous intercourse, which may cause increased intraabdominal pressure in the female partner and may tend to make the cul-de-sac tense and lessen the elasticity of the posterior fornix during deep penetration; (3) coital positions in which unusually deep penetration is experienced, such as the dorsal decubitus position with hyperflexion of the thighs and the sitting positions; (4) vaginismus, which may cause injury by shortening and narrowing of the vaginal tube; (5) genital disproportion, particularly in prepubertal girls and young women at first intercourse; (6) postmenopausal atrophy caused by increased friability and shortening of the vagina; (7) genital operations or injuries with disruption of the vaginal vault suture lines and/or shortening of the vagina; (8) uterine retroversion; and (9) rape.[5, 6, 17]

Lower vaginal injuries are more often seen in young women as a result of rape or first intercourse whereas tears of the vaginal vault are usually found in multiparous women. Both of these injuries can be very severe and cause perforation, subsequent bleeding in the peritoneal cavity, and even death.[4, 5, 7]

Only five cases of hemoperitoneum without perforation were found in the medical literature. Three of these were summarized by Engel[7] and included two lacerations of the left broad ligament and a higher parametrial laceration. Madej et al.[11] reported the fourth case, which was associated with a rupture of a corpus luteum cyst. A case report of bleeding caused by adhesive disease after coitus was reported in this country in 1986.[15] An interesting case of postcoital pneumoperitoneum 2 months after abdominal hysterectomy has been reported by Apesos et al.[14]

Dickinson[18] describes female genital injuries as being more common on the right because of dextrorotation of the uterus and an increased distensibility of the vagina in this area. Indeed three of the five cases reported here involved injuries on the right. It is conceivable that a combination of pelvic adhesions and vigorous deep thrusting could cause the injuries reported, but adhesions were present in only one of five patients reported on in this series. The cause of these other injuries remains obscure. In our cases no patient could recall any trauma during coitus that might have accounted for these intraabdominal injuries. Nevertheless practitioners should have a high index of suspicion of coital trauma even when examination of the vagina and external genitalia is unrevealing. While the incidence of postcoital bleeding from ligament or ovarian damage may, in fact, be rare, it is likely that postcoital trauma resulting in hemoperitoneum in association with rupture of corpus luteum cysts, ectopic pregnancies, and other ovarian lesions has previously been underreported.

REFERENCES

1. Blair R. Fatal injury of the vagina. Br Med J 1925;1:828.
2. Speert H. Vaginal lacerations resulting from coitus. AM J OBSTET GYNECOL 1947;53:177.
3. Speert H. Vaginal lacerations resulting from coitus. AM J OBSTET GYNECOL 1947;53:539.
4. Diddle AW. Rupture of vaginal vault during coitus. West J Surg 1948;56:414-6.
5. Fish SA. Vaginal injury due to coitus. AM J OBSTET GYNECOL 1956;72:544-8.
6. Chandra SP, Rather SD. Coital injuries (a review of 10 cases). Obstet Gynaecol (India) 1946;14:930-4.
7. Engel K. Uber Kohabitationsverletzungen, insbesondere mit intraperitonealen Lasionen and Blutungen. Zentrabl Gynaekol 1965;87:1189-95.
8. Krukierek S. Etiology of lesions of female genital organs during coition. Gynaecologia 1955;140:9-18.
9. Zeffer J. Peritoneumo athatolo hastso huvelyboltozati coitus-serules. Orv Hetil 1966;107:1238-9.
10. Kaiser P. Uber einen Fall von isolieter peritonealer Kohabitationsverletzung ohne Scheidenlason. Zentralbl Gynaekol 1968;90:1329-30.
11. Madej J, Urbanczyk C, Marczynski K. Coitus as a trauma causing rupture of the menstrual corpus luteum with massive intravisceral hemorrhage. Przegl Lek 1970;26:327-8.
12. Friedel W, Kaiser IH. Vaginal evisceration. Obstet Gynecol 1975;45:315-9.
13. Hall BD, Phelan JP, Pruyn SC, Gallup DG. Vaginal evisceration during coitus: a case review. AM J OBSTET GYNECOL 1978;131:115-6.
14. Apesos J, Srivastava JK, Williams C, Patton RJ. Postcoital pneumoperitoneum. Obstet Gynecol 1980;55:47S-9S.
15. Ferrara BE, Murphy WM Jr. The pains of love: hemoperitoneum following sexual intercourse. JAMA 1986; 255:1708-9.
16. Neugebauer L. Erreur de sexe. Pam Towarz Lik Warszaw 1895;91:369.
17. Rahm I. Ueber die Verletzung de Geburtsteila sub coitu. Acta Obstet Gynecol Scand 1927;6:28-58.
18. Dickinson RL. Atlas of human sex anatomy. Baltimore: Williams and Wilkins, 1949.

Postmortem chorionic villus sampling is a better method for cytogenetic evaluation of early fetal loss than culture of abortus material

Mark Paul Johnson, MD, Arie Drugan, MD, Frederick C. Koppitch III, MS, Wendy R. Uhlmann, MS, and Mark I. Evans, MD

Detroit, Michigan

In utero chorionic villus sampling at the time of diagnosis of intrauterine fetal death is compared with more traditional use of cultured fetal skin, products of conception, or amniocentesis. A total of 102 specimens from early fetal losses were evaluated for success in karyotyping and chromosomal results. We found postmorten chorionic villus sampling is technically possible, offers the highest likelihood of getting a cytogenetic result, and is a rapid, reliable, and safe technique. The extraembryonic component of intrauterine fetal deaths appears to remain viable and continues to grow long after the embryo has died. Samples obtained at the time of diagnosis of fetal death offer the greatest changes of successfully obtaining a karyotype. The incidence of chromosome abnormalities associated with fetal loss, particularly trisomies, is higher than previous data suggested. (AM J OBSTET GYNECOL 1990;163:1505-10.)

Key words: Abortion, pregnancy loss, chorionic villus sampling, amniocentesis

The degree of embryonic and fetal wastage in human beings has been found to be high. Several studies have suggested that 50% to 80% of fertilized ova are lost before reaching the second trimester. Chromosomal abnormalities are a significant factor in fetal loss, and rates of occurrence vary. Hook et al.,[1] predicted that at least 5% of conceptions as early as 5 weeks could be expected to be karyotypically abnormal. Warburton et al.,[2] postulated that 10% to 50% of all human conceptions may have an identifiable chromosomal defect.

Cytogenetic evaluation of embryonic or fetal loss has been hindered in the past by the poor success rate of obtaining viable cells for culture and successfully ob-

From the Division of Reproductive Genetics, Departments of Obstetrics and Gynecology and Molecular Biology and Genetics, Center for Molecular Biology, Hutzel Hospital/Wayne State University.
Received for publication January 9, 1990; revised June 19, 1990; accepted July 27, 1990.
Reprint requests: Mark Paul Johnson, MD, Division of Reproductive Genetics, Department of Obstetrics and Gynecology, Hutzel Hospital/Wayne State University, 4707 St. Antoine Blvd., Detroit, MI 48201.
6/1/24149

Table I. Reports studying spontaneous fetal losses

Reference	*Origin of specimens*	*Successfully karyotyped (%)*
Mueller et al.[16]	Fetal skin	47
Ellis and Bain[17]	Amnion	77
Angell et al.[18]	Amnion	69
Saal et al.[19]	Amniocentesis	100
Guerneri et al.[20]	Chorion villi	94

taining karyotyping. Successful karyotypes are obtained in spontaneously aborted tissues only approximately 50% of the time.[2] Other tissue sources were used from spontaneous abortions with improved success (Table I). These data suggest that the extraembryonic compartment may remain viable for a period of time after embryonic or fetal loss, and that those were the tissues that were most likely to be successfully cultured.

Along with other prenatal centers, we have been frustrated by the poor success rates in obtaining karyotypes in cases of fetal loss. Thus we began to try and herein report on the use of in utero chorionic villus sampling at the time of diagnosis of intrauterine fetal death as compared with the more traditional use of cultured fetal skin, products of conception, or amniocytes obtained by amniocentesis in cases of fetal loss.

Material and methods

Fetal karyotyping in 76 cases of fetal loss diagnosed by ultrasonographic visualization between 8 and 18 weeks' gestation was attempted between January 1987 and September 1989. A total of 33 skin biopsy specimens were obtained, 19 from previously expelled spontaneous abortions and 14 from fetal tissues obtained at the time of evacuation of intrauterine fetal deaths. Thirty-six specimens contained products of conception from which placenta and fetal membranes were cultured. All biopsy and products of conception specimens were placed into culture media immediately. All specimens from spontaneous abortion were obtained within 1 hour after delivery, inasmuch as most occurred on route to or in the emergency room. Seven amniocentesis procedures were done at the time of diagnosis of intrauterine fetal death.

From August 1988 to September 1989, 62 patients who had been referred to the Division of Reproductive Genetics at Hutzel Hospital were found to have intrauterine fetal death during detailed ultrasonographic examination before planned chorionic villus sampling. All patients with intrauterine fetal deaths were offered transcervical chorionic villus sampling on an experimental basis to determine whether a chromosomal cause could account for the fetal death. Of the 62 patients offered chorionic villus sampling on this basis, 23 (37.1%) chose to have the chorionic villus sampling performed. All attempts to obtain villi were successful by the transcervical approach, and yielded specimens ranging in size from 0.5 to 50.0 mg.

Specimens were processed by either a direct method or a long-term culture method involving mincing the villi or tissue specimen and culturing in an open system with Chang medium (#T100-058, Irvine Scientific, Santa Ana, Calif.) in T-25 tissue culture flasks (#25100, Corning Glass Works, Corning, N.Y.). All specimens were carefully examined and cleaned under a dissecting microscope before culturing to minimize the risk of maternal cell contamination. The long-term explants were then grown out in monolayer and harvested in suspension after trypsinization. Slides were made at room temperature immediately from cells pelleted and resuspended in 3 : 1 methanol/acetic acid solution. Twenty cells were then analyzed and 5 karyotyped after QFQ and GTG banding. If the sample size was >5 mg, then culturing and a direct method for analysis of mitotic figures were used. The direct method was undertaken on the basis of a modification of Simoni et al.[3] that uses 1% sodium citrate as the hypotonic, an open system with Chang medium, and a 45% to 50% acetic acid solution for cell dispersion. Slides were all prepared with a gravity flow method as described by Bhatia et al.[4] No inconsistencies were noted between results obtained by the short- or long-term methods, and karyotypes reported herein reflect long-term culture results.

Results

Of the 19 skin biopsy specimens from spontaneous abortions, 12 (63.2%) failed to grow. The exact time between delivery and placement in culture was not available from records; therefore the relationship between this variable and success in culturing could not be evaluated. Any specimen obtained more than 1 hour after expulsion was not included in this data. Seven successfully resulted in karyotypes of which four (57.1%) were normal and three (42.9%) were abnormal (69,XXY; 47,XY,+21; 47,XY,+21). In the 14 skin biopsy specimens obtained after evacuation of intrauterine fetal deaths, 12 failed to give results (95.7%) and the remaining two successfully karyotyped were abnormal (45,X/47,XX+21; 46,XX,+t21/21) (Table II).

Cultures from products of conception were more successful; only 13 (36.1%) failed to give results. Of the remaining successfully karyotyped specimens 18 (78.3%) had normal chromosomal complements, whereas five (21.7%) were abnormal (47,XXY; trisomy 9, 13, 16, 21).

Postmortem amniocentesis and postmorten chorionic villus sampling were both found to be technically feasible. The seven amniocentesis cultures resulted in

Table II. Cytogenetic evaluation of intrauterine fetal loss

Specimen	No.	Failure to grow		Normal		Abnormal		Karyotype
		n	%	n	%	n	%	
Skin								
Spontaneous abortion	19	12	63.2	4	57.1	3	42.9	69,XXY; 47,XY,+21; 47,XY,+21
Intrauterine fetal death	14	12	95.7	None	None	2	100	45,X/47,XX,+21; 46,XX+t(21/21)
Products of conception	36	13	36.1	18	78.3	5	21.7	47,XXY; Trisomy 9, 13, 16, 21
Amniocentesis	7	4	57.2	2	66.7	1	33.3	45,X
Chorionic villus sampling	26	None	None	2	7.7	24	92.3	See Table III
TOTAL	102	41	40.2	26	43.5	35	56.5	

Table III. Maternal age, technique, and karyotype

	No.	Mean maternal age (yr)	p Value
Total specimens	58	34.55 ± 5.50	—
All*			
Normal karyotype	25	29.25 ± 4.75	0.0018
Abnormal karyotype	33	36.67 ± 4.24	
Products of conception, total specimens	32	31.25 ± 4.71	0.0008
Chorionic villus sampling, total specimens	23	39.00 ± 3.38	
Chorionic villus sampling, abnormal karyotype	22	37.79 ± 3.43	0.0214
Products of conception, abnormal karyotype	10	33.71 ± 4.54	0.0825
Products of conception, normal karyotype	22	29.33 ± 4.09	

POC, Products of conception (includes all skin biopsy specimens).
*Includes amniocentesis results.

four (57.2%) growth failures, two (66.7%) normal karyotypes, and one (33.3%) abnormal 45,X karyotype. All 23 chorionic villus sampling specimens were successfully karyotyped, and 22/23 (95.7%) were abnormal. Abnormalities included one 69,XXX; one 69,XXY; three 45,X; one 49,XX,+18,+21,+22; one 46,XX,t(13q14q),+17; and trisomies of chromosome 13, 15 (x2), 16 (x3), 18, 20 (x2), 21 (x3), and 22 (x3).

Overall, the rate of successful growth and karyotyping of all embryonic death tissue was 58.6%. A total of 56.9% of cases were chromosomally abnormal with 71% trisomies, 12.9% 45,X, 9.6% triploidy, 3.2% mosaics, and 3.2% with multiple trisomies. The remaining 43.1% of cases had normal karyotypes. The mean maternal ages for cases with a normal karyotype with the use of the combined data were 29.25 years old, and 36.67 years old for the abnormal karyotype group ($p = 0.0018$) (Table III).

In all cases of skin specimens and products of conception after uterine evacuation for missed abortion, the clinical history was that of growth retardation that led to further evaluation. No histories for recurrent spontaneous miscarriage or fetal losses were found. The mean maternal age of those cases with normal karyotypes in the skin biopsy specimen and products of conception group was 29.33 years, whereas that for the abnormal karyotype group was 33.71 years (p = NS). Unfortunately, in most of these cases the gestational age was not listed, but the range for those in which gestational age was given ranged from 16 to 20 weeks. In the amniocentesis group the maternal ages for those with normal karyotypes were 30 and 21 years old at gestational ages of 19 and 16 weeks, respectively. The abnormal karyotype was from a 30-year-old woman at gestational week 14.

Successful growth and karyotyping of chorionic villus sampling intrauterine fetal deaths was 100%, of which 95.7% had abnormal chromosomal complements

Table IV. Postmortem chorionic villus sampling

*Maternal age (yr)**	*Gestational age (wk)†*	*Karyotype*
36	11	[46,XY]
35	10	[46,XY]
38	9	45,X
32	10	45,X
26	10	45,X
39	9.5	47,XX,+13
40	10	47,XX,+15
42	10	47,XX,+15
38	11	47,XX,+16
39	10	47,XX,+16
39	11	47,XY,+16
36	10	47,XY,+16
40	10	46,XX,t(13q,14q),+17
42	10	47,XX,+18
39	11.5	47,XX,+20
41	10	47,XY,+20
37	9	47,XY,+21
38	18	47,XX,+21
39	10	47,XX,+21
37	10	47,XX,+22
37	10	47,XX,+22
40	10	47,XX,+22
40	11	49,XX,+18,+21,+22
37	11	46,XY/47,XY,+15/47,XY,+21
36	11	69,XXY
35	10	69,XXX

*Mean maternal age = 37.87 ± 3.38 years.
†Mean gestational age = 10.52 ± 1.76 weeks.

(Table IV). The rate of trisomy was 69.56%, 45,X 13.0%, triploidy 8.7%, multiple trisomies 4.3%, and there were no mosaics detected. The most prevalent trisomies were of chromosome 16, 21, and 22 (13.0% overall, 18.8% of chorionic villus sampling trisomies), followed by chromosome 15 and 20 (8.7% overall, 12.5% of chorionic villus sampling trisomies). The single normal result came from a 36-year-old woman at 11 weeks' gestation. The mean maternal age for the abnormal chorionic villus sampling group was 37.87 years and mean gestational age was 10.52 weeks.

The maternal ages of all patients in the skin biopsy specimen and products of conception group were significantly younger than all patients in the chorionic villus sampling group ($p < 0.001$). When the maternal ages were compared for only those with *abnormal* karyotypes in these groups, the skin biopsy specimen–products of conception group was again significantly younger ($p = 0.0214$). The maternal ages of *all* patients with normal karyotypes (29.25 years) were also found to be younger when compared with all patients with abnormal karyotypes (36.67 years, $p = 0.0018$).

Comment

The high incidence of human fetal wastage is a challenging and complex problem. With statistical modeling, Roberts and Lowe[5] postulated a 78% loss rate of all human conceptions, most occurring even before the first missed menstrual period. With radioimmunoassay techniques for quantitative levels of β-subunit human chorionic gonadotropin, Edmonds et al.[6] followed up 118 pregnancies diagnosed on a biochemical basis prospectively and found 67 had elevated human chorionic gonadotropin levels without clinically obvious pregnancy; six of the remaining 61 pregnant women had clinical abortions, for a postimplantation loss rate of 62%. In a study of 34 early fertilized ova from 1 to 17 days, Hertig and Rock[7] found 13 had major abnormalities. In an earlier study, Hertig and Sheldon[8] in a morphometric study of 1000 spontaneous abortions found 75% to have major malformations. Other pathologic studies on abortuses by Mikano[9] and by Miller and Poland[10] supported Hertig's findings and showed the frequency of abnormalities was related to developmental stage. In these studies, 90% to 100% of specimens showed malformations if pregnancy losses were within 4 weeks after fertilization, and the frequency decreased when embryonic death occurred later in gestation.

Such information encouraged the search for genetic causes of pregnancy loss. Kajii et al.[11] studying elective terminations of pregnancy found a 4.5% incidence of chromosome abnormality in 469 successfully analyzed abortuses at 4 to 9 weeks' gestation. Yamamoto and Watanabe[12] found a 6.4% incidence of chromosome abnormalities in 1250 abortuses at 3 to 10 weeks' gestation. They further noted that the percentage was higher the earlier the developmental age of the embryo (9.3% at 3 to 4 weeks, 5.4% at 9 to 10 weeks). Warburton[13] noted that about 50% of chromosomally abnormal fetuses are lost between 8 to 15 weeks' gestation, about 24% in the period 16 to 19 weeks, and about 12% of abnormal fetuses are subsequently lost after 20 weeks.

In 62 patients scheduled to undergo routine prenatal testing, an intrauterine fetal death was diagnosed at the time of routine pretesting ultrasonographic examination. In the 23 patients who elected to undergo chorionic villus sampling, the villi were easily obtained by transvaginal approach, readily grew in culture, and were easily karyotyped. No maternal medical complications occurred as a result of this procedure. Karyotype results were available in 4 to 7 days for chorionic villus sampling specimens in contrast with the usual 2 to 3 weeks for skin, amniocentesis, or products of conception results.

Only one of the 23 specimens gave a normal chromosomal karyotype. There are several explanations for this, with differences in maternal age most siginficant. When *all* skin specimens and products of conception cases are compared with *all* chorionic villus sampling cases, the differences in maternal ages were signifi-

cant (skin–products of conception mean maternal age = 31.25 ± 4.71 years; chorionic villus sampling = 37.79 ± 3.38, $p = 0.0018$). When these data were corrected only for those with *abnormal* karyotypes (skin–products of conception = 33.71 ± 4.54; chorionic villus sampling = 37.87 ± 3.43, $p = 0.0214$), the results were again found to be statistically significant. If the maternal ages of *all* cases with abnormal karyotypes are compared with *all* those with normal karyotypes, the difference is again significant (abnormal = 36.76 ± 4.24 years, normal = 29.25 ± 4.75 years, $p = 0.0018$).

These findings are not unexpected on the basis of the increase in incidence of aneuploidy with advancing maternal age. In fact, 63.6% of the chorionic villus sampling specimens were trisomies. The most common trisomies were in agreement with Warburton's[13] findings in which trisomy 16 was most prevalent, followed by trisomy 21 and 22 (each representing 13% of chorionic villus sampling abnormal karyotypes), followed by trisomy 15 and 20 (8.7%) and then by 13, 17, 18 (4.3%). The 45,X complements were found in 13.6% and triploidy in 8.7%. Reportedly, 45,X is one of the most frequent chromosomal abnormalities found in early pregnancies, and yet only 13.6% of our chorionic villus sampling abnormal karyotypes had this chromosomal complement. Our data agree with other studies that have likewise found a negative association between incidence of 45,X karyotype and advancing maternal age.

Another possible explanation to the high incidence of abnormal chorionic villus sampling karyotypes could be the age at diagnosis. Fetal skin and products of conception specimens generally came from gestations of 14 to 18 weeks, whereas the chorionic villus sampling specimens were obtained at 9 to 11 weeks' gestation (mean = 10.52 ± 1.76 weeks). As noted earlier, the earlier a loss occurs in gestation, the greater the likelihood of an abnormal karyotype.

Last, chromosomal mosaicism might play a role in length of survival of pregnancies. Trophoblastic mosaicism is not uncommon in chorionic villus sampling specimens, but the absence of such in our cases was somewhat a surprise. It has been suggested recently[15] that survivability of anomalous gestations may depend on the presence of mosaicism in the extraembryonic compartment. In a study of trisomy 18 gestations, they suggest that cases that had a large or totally normal diploid component to the extraembryonic compartment might be advantaged, survive longer into gestation, and possibly represent the selective subset of anomalous pregnancies that survive to term. This concept might help explain the higher percentage of normal karyotypes when amnion or trophoblast was sent from intrauterine fetal deaths diagnosed and evacuated or miscarried later in gestation (as in our products of conception data). It may also determine when pregnancy loss occurs, and undoubtedly reflects the impact of each chromosomal abnormality on the ongoing development and differentiation of that particular gestation. This is likely why some chromosome anomalies are never seen in recognized pregnancies, and probably leads to developmental arrest very early in pregnancy. Those chromosome abnormalities (i.e., trisomy 16, 21, and 22), which occur more frequently in recognized pregnancy loss, probably represent a lesser impact on genomically directed embryogenesis and development, allowing more advanced gestational ages to be achieved. We believe that chorionic villus sampling done at the time of diagnosis of an intrauterine fetal death offers the greatest chance of obtaining a karyotype of that gestation. If such an approach is not possible, then a specimen that comprises chorionic villi and adjacent membranes (chorion frondosum) obtained temporally as close as possible to the time of diagnosis may represent a reasonable alternative.

REFERENCES

1. Hook EB. The impact of aneuploidy upon public health: mortality and morbidity associated with human chromosome abnormalities. In: Baughn-Dellarco V, Voytek P, Hollaender A, eds. Aneuploidy: etiology and mechanisms. New York: Plenum Press, 1985:7-34.
2. Warburton D, Kline J, Stein Z, Hutzler M, Chin A, Hassold T. Does the karyotype of a spontaneous abortion predict the karyotype of a subsequent abortion? Am J Hum Genet 1987;41:465-83.
3. Simoni G, Brambati B, Donesino C, et al. Efficient direct chromosome analysis and enzyme determinations from chrionic villi samples in the first trimester of pregnancy. Hum Genet 1983;63:349-57.
4. Bhatia R, Koppitch FC, Sokol RJ, Evans MI. Improving the yield in direct chorionic villus slide preparation. AM J OBSTET GYNECOL 1986;154:408-11.
5. Roberts CJ, Low CR. Where have all the conceptions gone? Lancet 1975;1:498-99.
6. Edmonds DK, Lindsay KS, Miller JF, Williamson E, Wood RJ. Early embryonic mortality in women. Fertil Steril 1982;38:447-53.
7. Hertig AT, Rock J. A series of potentially abortive ova recovered from fertile women prior to the first missed menstrual period. AM J OBSTET GYNECOL 1949;58:968-93.
8. Hertig AT, Sheldon WH. Minimal criteria required to prove prima facie case of traumatic abortion or miscarriage. Ann Surg 1943;117:596-606.
9. Mikano K. Anatomic and chromosomal anomalies in spontaneous abortions. AM J OBSTET GYNECOL 1970; 103:143-54.
10. Miller JR, Poland BJ. The value of human abortuses in the surveillance of developmental anomalies. Can Med Assoc J 1970;103:501-2.
11. Kajii T, Ohama K, Mikamo K. Anatomic and cytogenic studies of 944 induced abortions. Hum Genet 1978; 43:247-58.
12. Yamamoto M, Watanabe G. Epidemiology of gross chromosome anomalies at the early embryonic stage of pregnancy. Contrib Epidemiol 1979;1:101-6.

13. Warburton D. Chromosomal causes of fetal death. Clin Obstet Gynecol 1987;30:268-77.
14. Wright DJ, Brindley BA, Koppitch FC, Drugan A, Johnson MP, Evans MI. Interpretation of chorionic villus sampling laboratory results is just as reliable as amniocentesis. Obstet Gynecol 1989;74:739-74.
15. Kalousek DK, Barrett IJ, McGillivray BD. Placental mosaicism and intrauterine survival of trisomies 13 and 18. Am J Hum Genet 1989;44:338-43.
16. Mueller RF, Sybert VP, Johnson J, Brown ZA, Chen WJ. Evaluation of a protocol for post-mortem examination of stillbirths. N Engl J Med 1983;309:586-90.
17. Ellis PM, Bain AD. Cytogenetics in the evaluation of perinatal death. Lancet 1984;1:630.
18. Angell RR, Sandison A, Bain AD. Chromosome variation in perinatal mortality: a survey of 500 cases. J Med Genet 1984;21:39-44.
19. Saal HM, Rodis J, Weinbaum PJ, DiMaggio R, Landrey TM. Cytogenetic evaluation of fetal death: the role of amniocentesis. Obstet Gynecol 1987;70:601-3.
20. Guerneri S, Bettio D, Simoni G, Brambati B, Lanzani A, Fraccaro M. Prevalence and distribution of chromosome abnormalities in a sample of first trimester internal abortions. Hum Reprod 1987;2:735-9.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Detection of congenital toxoplasmosis by chorionic villus sampling and early amniocentesis

Walter Foulon, MD,[a] Anne Naessens, MD,[b] Luc de Catte, MD,[a] and Jean-Jacques Amy, MD[a]

Brussels, Belgium

We describe a strategy used in this case to detect congenital toxoplasmosis early in pregnancy. Combination of chorionic villus sampling and early amniocentesis with an in vitro cell culture technique makes prenatal diagnosis of congenital toxoplasmosis possible from the end of the first trimester of pregnancy. (Am J Obstet Gynecol 1990;163:1511-3.)

Key words: Congenital toxoplasmosis, early prenatal diagnosis

Recently the use of cordocentesis for detection of congenital toxoplasmosis in the second trimester of pregnancy[1] was described. Diagnosis of congenital toxoplasmosis is made on the basis of the presence of specific immunoglobulin M (IgM) antibodies and the isolation of the parasite by means of mouse inoculation. Moreover, elevated liver enzymes, low platelet counts, and elevated eosinophils in fetal blood are aspecific markers indicative of fetal infection. Although the procedure of fetal blood sampling is relatively safe it can only be offered from week 19 onward. When seroconversion occurs early in pregnancy or in cases of high initial antibody level in the first trimester of pregnancy one must wait until the moment of fetal blood sampling to know the results of the aspecific indexes of congenital toxoplasmosis. The results of the mouse inoculation test are only available after 4 to 5 weeks. Therefore it seems important to develop an earlier and accurate prenatal diagnosis to reduce the anxiety inherent to the long period of waiting. Moreover, early detection enables termination of the pregnancy or the start of treatment with pyrimethamine and sulfadiazine in cases of proved congenital toxoplasmosis at a much earlier stage.

Case report

A 26-year-old woman was initially seen at 8 weeks' gestation. During routine serologic screening high levels of antibodies against *Toxoplasma gondii* were found. The titer of the Sabin-Feldman dye test was 1/4000, that of the complement fixation test was 1/16, and that of the specific IgM was 1/80. Ultrasonographic examination showed a conceptus with a heart beat and a crown-rump length of 18 mm. The patient was informed of the risk of giving birth to a congenitally infected infant and consented to a prenatal diagnosis at 19 weeks' gestation. Treatment with spiramycin (Rovamycine, Rhône-Poulenc, Paris) 3 gm daily was started from week 9 onward.

A second ultrasonographic scan at 14 weeks' gestation revealed the fetus had died. Before terminating the pregnancy a transabdominal chorionic villus sampling was performed. Ten milligrams of pure placental tissue was aspirated. Amniotic fluid samples were taken immediately thereafter. The chorionic tissue and 3 ml amniotic fluid were used for in vitro culture of *Toxoplasma gondii* on fibroblasts.[2] A second aliquot of 3 ml of amniotic fluid was inoculated into mice and the remaining 20 ml was cultured for determination of the fetal karyotype. The pregnancy was terminated by extraamniotic administration of prostaglandin. The patient expelled a dead fetus without signs of malformation. Fragments of placenta, fetal brain, liver, and lung were processed for detection of toxoplasma by tissue culture method[2] and by means of animal inoculation.

Toxoplasma gondii was identified by tissue culture in the chorionic villus sample (Fig. 1), the placenta, fetal brain, liver, lung, and amniotic fluid after 5 days. The parasite was also isolated after 5 weeks from mice inoculated with amniotic fluid, placenta, fetal brain, and lung. Mouse inoculation of fetal liver remained negative. A chorionic villus sample was not available for mouse inoculation. Histologic examination showed toxoplasma cysts in the fetal brain and placenta. The fetal karyotype was found to be 46,XY and normal.

Comment

An infection with *Toxoplasma gondii* during pregnancy may result in congenital disease. Approximately

From the Departments of Gynecology, Andrology, and Obstetrics,[a] and Microbiology,[b] Academisch Ziekenhuis, Vrije Universiteit Brussel.
Received for publication January 9, 1990; revised April 12, 1990; accepted June 2, 1990.
Reprint requests: Walter Foulon, MD, Department of Gynecology, Andrology, and Obstetrics, Academisch Ziekenhuis, Laarbeeklaan, 101, Brussels, Belgium B-1090.
6/1/22871

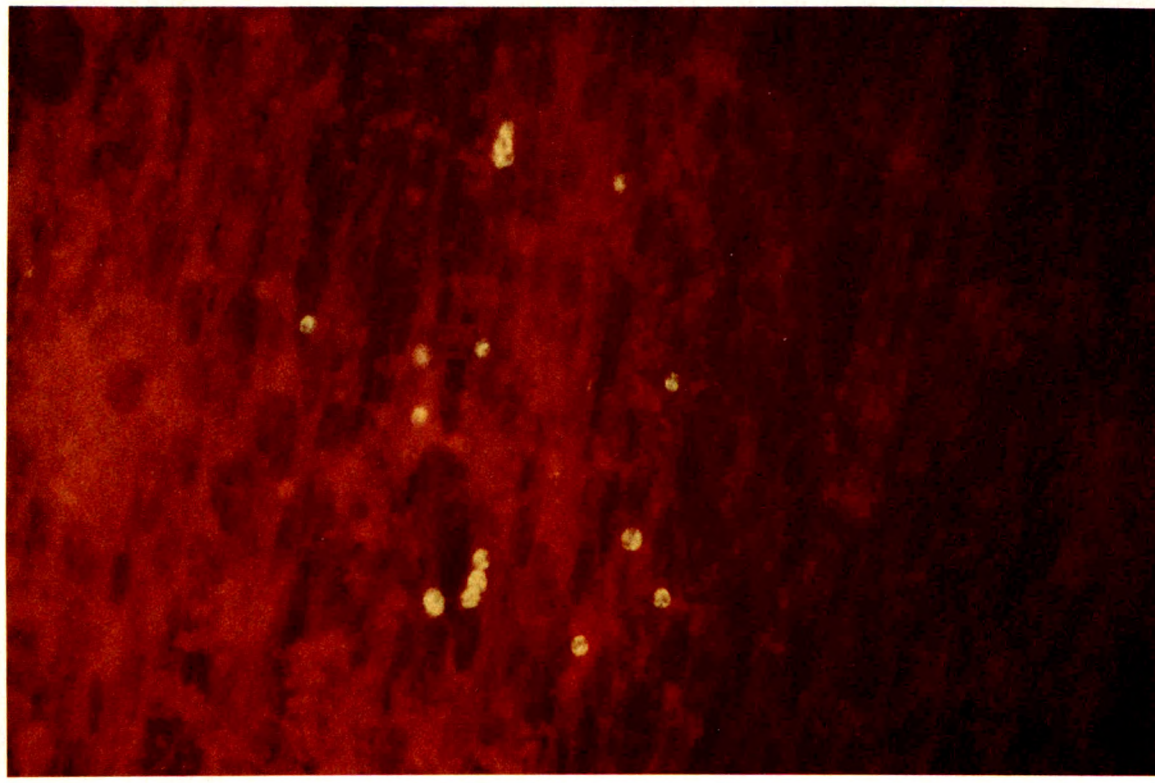


Fig. 1. In vitro cell culture of *Toxoplasma gondii*: Microscopic view of parasite after coloration with monoclonal antibody.

20% of actively infected women before 20 weeks' gestation transmit the parasite to their fetus.[1] Prenatal investigation should be limited to patients in whom seroconversion occurs during pregnancy or in women with high initial antibody levels suggestive of recent toxoplasmosis. Presence of IgM antibodies is not necessarily indicative of recent maternal infection. IgM activity can occasionally persist for some years after a toxoplasma infection or nonspecific reactions may occur. Moreover, some patients may exhibit naturally occurring IgM antibodies. Therefore one must be familiar with the interpretation of the toxoplasma serology in selection of a population at risk of having a congenitally infected fetus.

Prenatal diagnosis of congenital toxoplasmosis by means of cordocentesis is an important step forward in the management of toxoplasma infection during pregnancy. Nevertheless interpretation of the nonspecific tests is not always easy because of their low positive predictive value.[1] Specific signs of congenital toxoplasmosis, namely IgM antibodies, are found in only 21% of affected fetuses[1] and the result of the inoculation of amniotic fluid and fetal blood into mice takes 4 or 5 weeks. This means that the definitive result may not be known until 25 weeks' gestation.

The case we described illustrates that chorionic tissue culture and small amniotic fluid samples taken early in gestation can be used for the detection of congenital toxoplasmosis. Small placental biopsy specimens (10 mg of chorionic tissue) and 3 ml of amniotic fluid are apparently sufficient to detect the parasite in utero. Although a positive placenta culture is not always associated with fetal infection there is a close correlation between positive isolations and congenital infection. The positive predictive value of a positive placenta culture is at least 95%.[3] Combination of chorionic villus sampling and early amniocentesis with an in vitro cell culture technique makes prenatal detection of congenital toxoplasmosis theoretically possible at the end of the first trimester of pregnancy. It must be said that in this case the toxoplasma infection was overwhelming, leading to fetal death. It remains to be proved that chorionic villus sampling and early amniocentesis are as reliable as cordocentesis in the detection of congenital toxoplasmosis. Therefore chorionic villus sampling and early amniocentesis should not replace cordocentesis but rather should be used in association with cordocentesis until more is known about the sensitivity and specificity of diagnostic methods that use these techniques.

REFERENCES

1. Daffos F, Forestier F, Capella-Pavlovsky M, et al. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. N Engl J Med 1988;318:271-5.
2. Derouin F, Thulliez P, Candolfi E, Daffos F, Forestier F. Early prenatal diagnosis of congenital toxoplasmosis using amniotic fluid samples and tissue culture. Eur J Clin Microbiol Infect Dis 1988;7:423-5.
3. Desmonts G, Couvreur J. Toxoplasmosis: epidemiologic and serologic aspects of perinatal infection. In: Krugman S, Gershon AA, eds. Infections of the fetus and newborn infant. New York: Alan R Liss Inc, 1975:115.

The "stuck twin" phenomenon: Ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses

Barry S. Mahony, MD, Charles N. Petty, MD, David A. Nyberg, MD, David A. Luthy, MD, Durlin E. Hickok, MD, and Jack H. Hirsch, MD

Seattle, Washington

Thirteen consecutive twin pregnancies affected by the "stuck twin" phenomenon were reviewed to determine the potential benefit of serial amniocenteses. The fetal survival rate for the eight pregnancies that underwent serial amniocenteses was 69% (11 of 16 fetuses). This is significantly improved compared with a fetal survival rate of 20% among the five preceding pregnancies managed without serial amniocenteses at the same institution ($p = 0.01$). It is also markedly improved compared with a combined fetal survival rate of 16% among 48 previously reported pregnancies with the stuck twin phenomenon managed without serial amniocenteses ($p < 0.0001$). Survival correlated with the absence of concomitant pregnancy complications (i.e., maternal hypertension or intractable labor) and with the absence of severe fetal structural abnormalities. Procedural complications occurred in three of eight pregnancies (37.5%) managed with serial amniocenteses and was attributed as a cause of fetal death in one case. Two of 11 survivors (18%) had complications after serial amniocenteses including brain infarction and renal tubular necrosis. Serial amniocenteses may significantly improve the survival rate of twin gestations affected by the stuck twin phenomenon but may be associated with complications among survivors. (Am J Obstet Gynecol 1990;163:1513-22.)

Key words: Twins, stuck twins, polyhydramnios, oligohydramnios, serial amniocenteses

The "stuck twin" phenomenon has been described as a diamniotic pregnancy in which one fetus resides against the uterine wall in a severely oligohydramniotic sac and the co-twin is in a severely polyhydramniotic sac.[1] It complicates up to 8% of twin pregnancies and 35% of monochorionic diamnionic gestations, although it can also occur in dichorionic and even dizygotic pregnancies.[2-4] Some, perhaps most, cases appear to result from the twin transfusion syndrome, but this is not universally present in the stuck twin phenomenon. The severe disparity of amniotic fluid volume signals a dismal prognosis for both twins with most reports indicating a survival rate of <20%.[1, 3, 5-7] Premature labor develops in almost all cases, probably secondary to uterine distension.[3]

The extremely high mortality rate for these fetuses has stimulated a variety of proposed therapies including selective feticide,[2, 8] hysterotomy for umbilical cord ligation of one twin,[9] bloodletting from a placental vessel,[10] laser coagulation of placental anastomotic vessels, and serial amniocenteses.[2-4, 10-21] However, no comparison studies have been reported to indicate the utility of these management options.

This article describes our experience with regard to serial therapeutic amniocenteses in eight consecutive pregnancies affected by the stuck twin phenomenon and compares the fetal survival rate in these eight pregnancies with the fetal survival rate of the previous five affected pregnancies managed at our institution without serial amniocenteses. These results are also compared with prior reports of the stuck twin phenomenon managed with or without serial amniocenteses.

From the Divisons of Perinatal Medicine and Ultrasound, Swedish Hospital Medical Center.
Received for publication October 16, 1989; revised March 27, 1990; accepted July 27, 1990.
Reprint requests: Dr. Barry S. Mahony, Suite 1150, 1229 Madison St., Seattle WA 98104.
6/1/24148

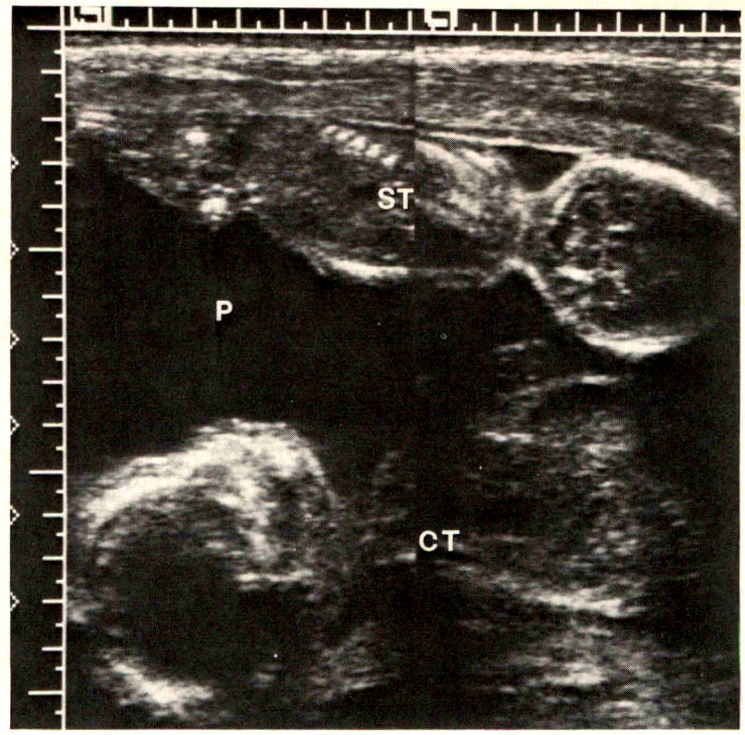


Fig. 1. Dual-image longitudinal ultrasonogram at 19.5 weeks' gestation shows stuck twin adjacent to anterior uterine wall (case 7). Although amnion separating the two fetuses is not apparent on this scan, its presence adjacent to stuck twin is inferred by relative fixation of this twin in nondependent position. Co-twin is in polyhydramniotic sac (additional scans showed a 13 cm vertical pocket of amniotic fluid). *ST*, Stuck twin; *P*, polyhydramnios; *CT*, co-twin in polyhydramniotic cavity.

Material and methods

Seventeen pregnancies with the stuck twin phenomenon were evaluated at this institution between January 1986 and June 1989. Four of these underwent elective second-trimester termination of pregnancy. The remaining 13 pregnancies were managed at this institution and form the basis for this report. In each case, tocolysis was provided as necessary with indomethacin, magnesium sulfate, or ritodrine. Patients were managed on an inpatient basis for preterm labor but were otherwise managed as outpatients. From 1986 through 1987 five of the pregnancies were managed without serial amniocenteses. The subsequent eight pregnancies from January 1988 through June 1989 were managed with serial amniocenteses.

In each case, one twin (the stuck twin) remained relatively fixed in position adjacent to the uterine wall even with changes in maternal position (Fig. 1). Small portions of the thin separating membrane were visible adjacent to the fetus, indicating that the fetus was in a severely oligohydramniotic sac of a diamniotic pregnancy (Fig. 2). The co-twin was in a moderately-to-severely polyhydramniotic sac in each case. We defined moderate-to-severe polyhydramnios as when the largest vertical pocket of amniotic fluid measured >12 cm by ultrasonography.[22] No structural abnormalities were evident on the initial ultrasonographic examination of each of the stuck twins, but two of the polyhydramniotic twins had sonographically detectable structural abnormalities (one case of esophageal atresia and one case of congenital heart disease).

Among pregnancies managed with serial amniocenteses, the amniocenteses were begun at the time of the initial diagnosis after detailed counseling regarding options and potential complications. Amniotic fluid was removed from the polyhydramniotic sac over a period of 45 to 90 minutes under continual ultrasonographic guidance with a 21 or 22 gm Hakko needle attached to a collecting tube. Fluid was removed until the volume of fluid in the polyhydramniotic sac was judged to be normal by ultrasonography (largest vertical pocket of amniotic fluid <7 cm).[22] After the initial amniocentesis was performed, this procedure was repeated when the ultrasonogram showed that the amniotic fluid had reaccumulated to moderate-to-severe levels of polyhydramnios.

We compared the fetal survival rate for the eight pregnancies in this series managed with serial amniocenteses with the fetal survival rate of the preceding five pregnancies managed at this institution without

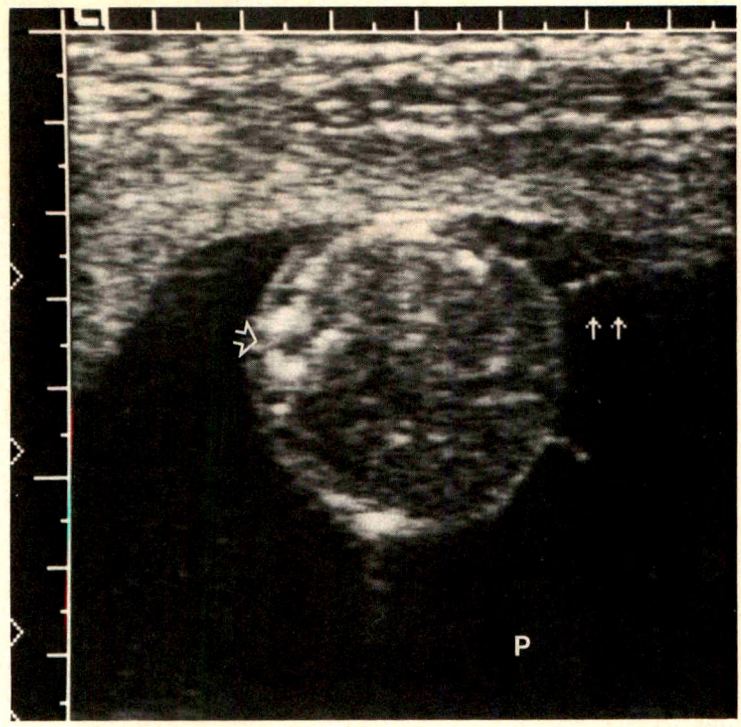


Fig. 2. Cross-section ultrasonogram of abdomen of stuck twin at 16 weeks' gestation shows small portion of amnion as it sweeps off fetus and approaches uterine wall. This indicates that fetus is in severely oligohydramniotic sac (case 8). Additional scans showed co-twin in polyhydramniotic cavity. *Arrows*, Amnion separating the stuck twin from the polyhydramniotic twin; *open arrow*, fetal spine; *P*, polyhydramnios.

serial amniocenteses. We also reviewed previous published reports of twin pregnancies managed with amniocenteses (n = 21) or without amniocenteses (n = 48). Statistical analysis was performed with the Fisher two-tailed exact test.

Results

Table I summarizes the clinical data, medical management, and outcome for the 13 pregnancies with the stuck twin phenomenon managed at this institution since 1986. Two of the 10 (20%) fetuses managed without amniocenteses survived including none of the stuck twins in the oligohydramniotic sac. One survivor occurred in a pregnancy in which its stuck co-twin died at 19 weeks' gestation (case 3). The pregnancy progressed to term without further complications and resulted in a normal neonate who remains well at 30 months. The other survivor in this set of pregnancies was delivered at 27 weeks' gestation because of intractable labor; at 29 months this survivor has chronic obstructive pulmonary disease and hypotonic cerebral palsy related to the extreme prematurity (case 5).

Eleven of the 16 fetuses (69%) managed with serial amniocenteses survived. Fetal death of one twin pair occurred at 24.5 weeks as a result of intractable premature labor and spontaneous rupture of membranes (case 13). Another twin pair died during the neonatal period after a pregnancy complicated by abruptio placentae at 21 weeks, severe maternal hypertension at 31 to 32 weeks, and congenital heart disease of the polyhydramniotic twin (case 12). One fetal death was attributed to a true cord knot that developed after serial amniocenteses (case 11). Two of the 11 (18%) survivors, both in an oligohydramniotic sac, had complications including brain infarction (cases 6 and 7; Fig. 3) and renal tubular necrosis (case 6; Fig. 4). One twin in a polyhydramniotic sac had moderate transient cardiomyopathy that occurred after 5 weeks of severe hydrops fetalis (case 10). Intracranial hemorrhage occurred in two neonates after premature delivery at 30 to 32.5 weeks (cases 7 and 10).

The maternal age and gestational age at the time of initial diagnosis was similar for pregnancies managed with and without serial amniocenteses. However, the gestational age at delivery was greater for the eight pregnancies managed with serial amniocenteses (median, 31 to 32 weeks; mean, 31.4 weeks) than for the preceding five pregnancies managed without amniocenteses (median, 25 weeks; mean, 26.6 weeks). Four of five pregnancies managed without serial amniocen-

Table I. Clinical data, medical management, and outcome of 13 pregnancies with the stuck twin phenomenon managed with or without serial amniocenteses

Case No.	Maternal age (yr)	Gestational age at diagnosis (wk)	Tocolysis	Gestational age at delivery (wk)	Stuck twin		Polyhydramniotic twin	
					Birth weight (gm)	Outcome	Birth weight (gm)	Outcome
1986 to 1987 (No serial amniocenteses)								
1	34	17.5	+	19	145	Death	220	Death
2	33	23.5	+	24	410	Death	490	Death
3	23	23	—	38	—	Death	2667	Alive and well at 30 mo
4	30	24.5	+	25	750	Death	920	Death
5	18	18	+	27	670	Death	870	Hypotonic cerebral palsy; COPD at 29 mo
1988 to 1989 (Serial amniocenteses)								
6	26	22	+	33	1220	Brain infarcts; CRF; DD at 15 mo	1950	Alive and well at 15 mo
7	35	17.5	+	30	1460	Brain infarcts; DD at 14 mo	1410	Neonatal ICH; slight DD at 14 mo
8	41	16	—	38	2295	Alive and well at 9 mo	2341	Alive and well at 9 mo
9	32	28.5	+	31	1550	Alive and well at 5 mo	1120	TEF; alive and well at 5 mo
10	30	20	+	32.5	1392	Neonatal ICH; mild hydrocephalus at 3 mo	1673	Resolved cardiomyopathy; alive and well at 3 mo
11	22	26	+	30	700	Death (true cord knot)	1500	Alive and well at 14 mo
12	25	20.5	+	32	840	Death	2700	Death; hypoplastic left heart
13	26	19	+	24.5	359	Death	440	Death

COPD, Chronic obstructive pulmonary disease; *CRF*, chronic renal failure (stable creatinine of 2 mg/dl); *TEF*, tracheoesophageal fistula with esophageal atresia (surgically repaired); *ICH*, intracranial hemorrhage; *DD*, developmental delay.

teses developed preterm labor and each of these was delivered before 27 weeks; one of these pregnancies progressed to term without tocolysis. Preterm labor developed in five of the eight pregnancies managed with serial amniocenteses and one pregnancy progressed to term without tocolysis.

Table II shows the prenatal data regarding the eight pregnancies managed with serial amniocenteses. The reaccumulation of amniotic fluid did not follow a discernable pattern and the intervals between amniocenteses varied markedly from case to case as well as within individual pregnancies. Procedural complications related to amniocenteses occurred in three of eight (37%) pregnancies and three of 28 (11%) serial amniocenteses. They included chorioamniotic separation (case 7), abruptio placentae (case 12), and rupture of the amnion (case 11). Rupture of the amnion with a subsequent true cord knot was attributed as a cause of one fetal death (case 11).

The fetal survival rate of 69% for the eight pregnancies managed with serial amniocenteses is significantly improved compared with a fetal survival rate of 20% for the five prior affected pregnancies managed without serial amniocenteses at this institution ($p = 0.01$). It is also markedly improved compared with the combined fetal survival rate of 15.6% in 48 previously reported affected pregnancies not managed with serial amniocentesis ($p < 0.0001$).[1, 3, 5-7, 23]

The fetal survival rate for the current series of pregnancies managed with serial amniocenteses is not significantly different from the combined fetal survival rate of 64% from previous reports of 21 twin pregnancies managed with serial amniocenteses ($p > 0.05$; Table III).[2-4, 10-21] Although the lack of prenatal

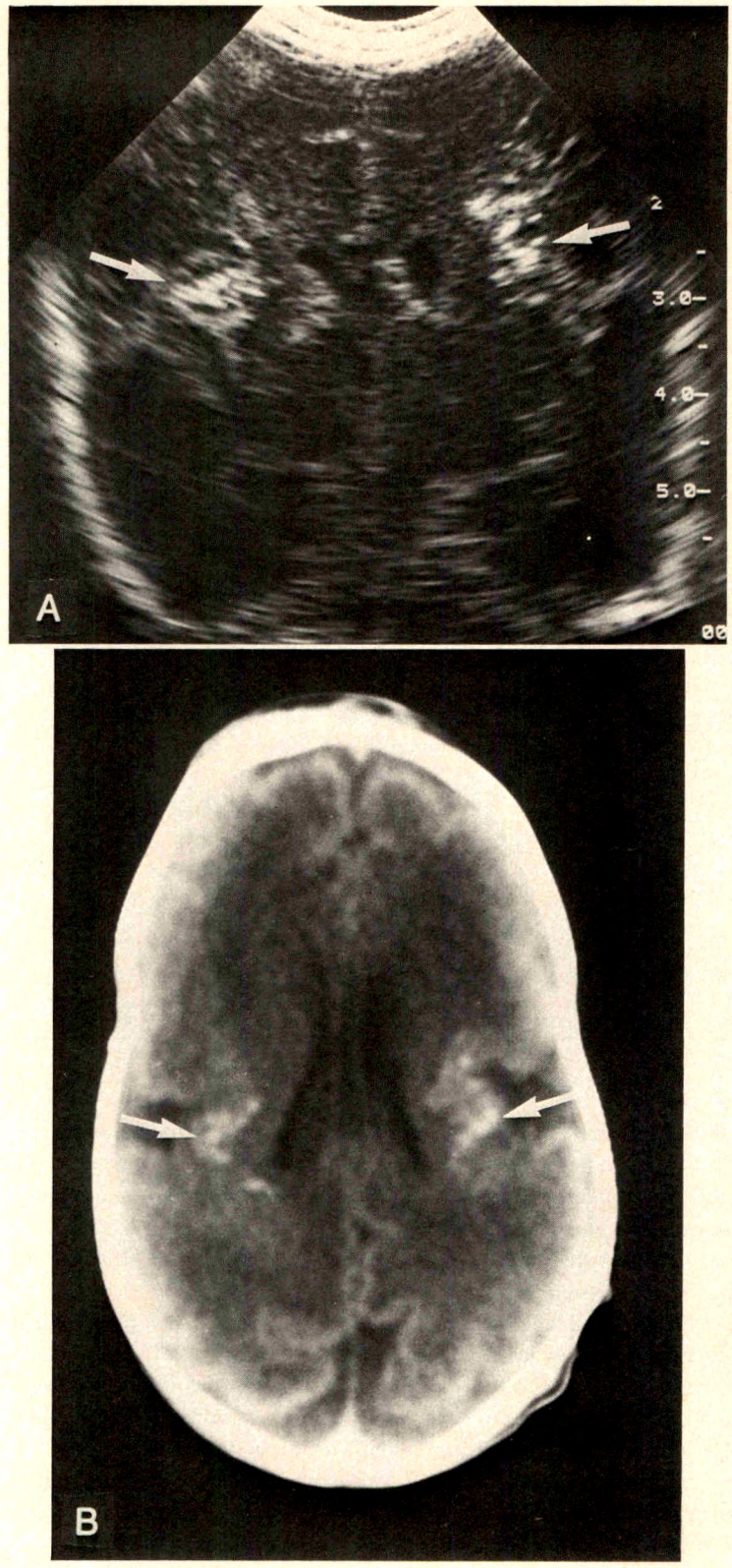


Fig. 3. Neonatal coronal head ultrasonogram (**A**) and axial head computed tomography (**B**) during first week after delivery at 33 weeks show bilateral calcifications in region of basal ganglia indicative of prior infarctions (case 6). This stuck twin had been managed with serial amniocentesis. After removal of 5.5 L of amniotic fluid at 24 weeks' gestation, fetus had transient hydrops fetalis and mild cerebral ventricular dilation lasting <1 week. *Arrows*, Calcifications in region of basal ganglia.

Table II. Timing and amount of serial amniocenteses and prenatal complications among eight pregnancies with the stuck twin phenomenon

	Amniocentesis		*Prenatal complications (wk)*		
Case No.	*Gestational age (wk)*	*Amount (L)*	*Stuck twin*	*Polyhydramniotic twin*	*Maternal; placental membranes*
6	24	5.5	Hydrops; cerebral ventricular dilatation; echogenic kidneys (24.5)	—	—
	30.5	2.5		—	—
	30.7	1.9		—	—
	31	2.0		—	—
7	19.5	1.8	—	—	Chorioamniotic separation (24); spontaneous rupture of membranes (27)
	23	2.2	—	—	
	24	3.0	—	—	
8	16	0.3	—	—	—
	17	0.5	—	—	—
9	28.5	1.0	—	—	—
	30	0.7	—	—	—
10	20.5	0.5	Hydrops (31.5) Amnionitis (32.5)	Hydrops (26.5-31.5)	—
	21.3	1.2			—
	26.5	0.9			—
	26.6	0.8			—
11	26	4.6	True knot in cord with death (29)	Hydrops (30)	Rupture of the amnion (28.5)
	28	2.0			
	28.5	5.0			
	29.5	2.5			
12	20.5	2.5	—	Hydrops (31-32)	Abruptio placentae (21) Preeclampsia (31-32)
	23.5	0.3	—		
	25	0.3	—		
13	18.8	1.2	—	—	Spontaneous rupture of membranes (24.5)
	19	0.9	—	—	
	20	0.9	—	—	
	21	1.5	—	—	
	22	1.2	—	—	
	23.5	1.5	—	—	

ultrasonography in early reports precludes confirmation of the stuck co-twin, some authors described an extreme paucity of amniotic fluid in one sac at the time of delivery.[17] One can reasonably presume that many of the early reported cases had the stuck twin phenomenon on the basis of the common occurrence of this phenomenon and the relative rarity of other causes for acute polyhydramnios in twin pregnancies.[24] Combination of the present series with prior reports yields a fetal survival rate of 65.5% among 29 affected pregnancies managed with serial amniocenteses compared with a significantly lower fetal survival rate of 15.6% for 48 affected pregnancies not managed with serial amniocenteses ($p < 0.00001$).

Comment

Marked disparity of amniotic fluid volume that arises acutely in a diamniotic twin pregnancy between approximately 18 and 30 menstrual weeks commonly indicates the stuck twin phenomenon.[1-3] The precise cause of the markedly disparate amniotic fluid volumes in the stuck twin phenomenon remains speculative. It undoubtedly results from the twin transfusion syndrome in some cases, as shown by significant disparity in twin size and by postnatal documentation of placental anastomoses.[23, 25-28] However, other causes must also be operative because the stuck twin phenomenon can occur in dichorionic and even dyzygotic pregnancies.[2-4] In addition, fetal hematocrit levels and umbilical arterial Doppler interrogation often yield variable and confusing findings that do not differentiate donor from recipient twin,[6, 11, 29, 30] and most twins with disparate hemoglobin levels (>5 mg/dl) and disparate birth weights (>20%) do not have the twin transfusion syndrome.[31] Furthermore, in some pregnancies affected by the stuck twin phenomenon either twin may have hydrops, but in other affected pregnancies the polyhydramniotic twin often lacks manifestions of hydrops and the stuck twin lacks evidence of placental insufficiency.

We suggest that diverse causes may initially produce polyhydramnios in one of the sacs of a diamniotic preg-

Table III. Literature review of serial amniocenteses in the management of twin pregnancies*

Case No.	Gestational age at diagnosis (wk)	Gestational age at delivery (wk)	Total fluid removal (liters)	No. of amniocenteses	Complications	Fetal survival		Reference
						Twin 1	Twin 2	
1	38	40	1.5	2	—	No	Yes	12
2	25	39	2.6	2	—	No	Yes	12
3	25	30	2.8	2	—	No	No	12
4	34	40	2.4	2	—	Yes	Yes	12
5	21	36	18.0	4	—	No	Yes	13
6	28	40	3.1	3	—	No	Yes	14
7	24	40	2.8	2	—	No	Yes	15
8	29	36.5	10.5	6	—	Yes	Yes	16
9	16	31	11.4	4	—	Yes	Yes	17
10	22	37	15.3	3	—	Yes	Yes	18
11	28	39	10.8	2	—	Yes	Yes	19
12	18	37	6-9	3	—	Yes	Yes	20
13	26	35	7.6	5	—	No	Yes	4
14	23	34	3.0	2	—	Yes	Yes	4
15	24	28.5	3.5	7	—	No	Yes	11
16	26.5	28	2.8	5	Labor, renal cortical necrosis	No	Yes	11
17	22.3	33.6	21.0	17	Cordocentesis	Yes	Yes	10
18	22.7	33.6	2.1	2	Cordocentesis	Yes	Yes	10
19	20	21	3.8	2	—	No	No	2
20	23	24	2.0	3	Labor, amnionitis	No	No	2
21	27.5	32.5	†	†	—	No	Yes	3
TOTAL	—	—	—	78	—	9/21	18/21	—
Mean	24.9	34	6.8	3.9	—	—	—	—
Median	24	36	3.8	3	—	—	—	—

*Does not include reference 21 because pertinent data not reported in original article.
†Data not reported in original article.

nancy and eventually result in the stuck twin phenomenon. Case 9, in which the small polyhydramniotic twin had a tracheoesophageal fistula and the stuck twin was structurally normal, represents a relatively unusual cause for the stuck twin phenomenon but exemplifies the effect of the polyhydramnios on the co-twin. The cause of the initial increase in fluid within one amniotic cavity remains unexplained by this hypothesis in cases without structural cause for polyhydramnios. However, once present it may result in increased atriopeptin secretion and cause a feedback mechanism, producing increased urine production and worsening polyhydramnios of the twin in the polyhydramniotic sac.[32-34]

Combined experience from various reports indicates that both twins are structurally normal in approximately 95% of pregnancies affected by the stuck twin phenomenon.[1-3, 5-8, 10, 23, 35] Although neither fetus usually has an identifiable anomaly, the marked disparity of amniotic fluid volume may result from anomalies that lead to polyhydramnios for one twin (including neural tube defect, upper gastrointestinal obstruction, congenital heart disease) or oligohydramnios for the other twin (ruptured amnion, urinary tract anomalies, asymmetric growth retardation).[36] In the current series, two of 13 (15%) affected pregnancies had fetal structural anomalies detected with prenatal ultrasonography, and both anomalous fetuses were in the polyhydramniotic sac. Therefore detection of the stuck twin phenomenon warrants a careful fetal survey of both twins.

Whatever the underlying pathogenesis for the stuck twin phenomenon, it carries an extremely high natural fetal mortality rate of >80%. This high mortality rate has prompted a variety of other proposed prenatal therapies in addition to serial amniocenteses. Selective feticide of the stuck twin was performed in two cases with one case resulting in birth of a normal co-twin. The other case resulted in death of the co-twin.[2, 8] However, the potential for embolism from the dead or dying twin to the live twin via placental anastomoses, as well as the ethical issues associated with selective feticide, limits the utility of this mode of therapy for stuck twins. Umbilical cord ligation has been attempted, although it has not yet been succcessful.[9] Since initially reported by Rivett in 1933, therapeutic amniocenteses have been used occasionally to treat acute polyhydramnios in twin pregnancies.[2-4, 10-21]

Our experience, supported by the composite literature, suggests that serial amniocenteses may provide an effective means of managing pregnancies with the stuck twin phenomenon. The survival rate for affected twin pregnancies at our institution improved significantly

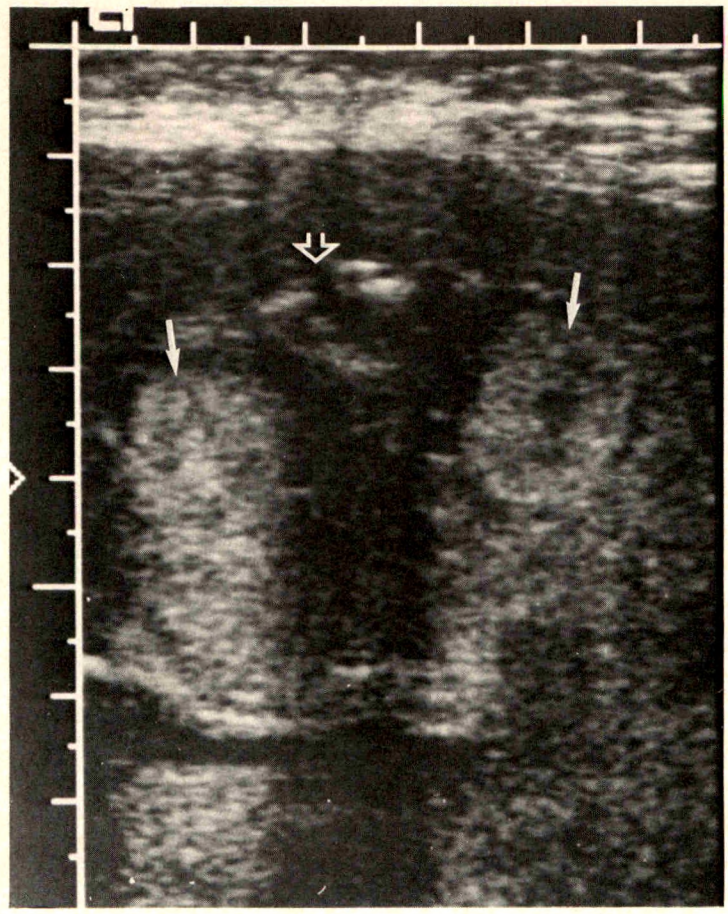


Fig. 4. Transverse ultrasonogram of stuck twin's kidneys at 24.5 weeks' gestation after removal of 5.5 liters of amniotic fluid from polyhydramniotic sac of co-twin, shows kidneys to have abnormally increased cortical echogenicity. This child has chronic renal failure with a stable creatinine value of 2 mg/dl at 15 months (case 6). *Arrows*, Echogenic kidneys; *open arrow*, spine.

from 20% to 69% with the introduction of serial amniocentesis. The survival rate now appears predominantly related to concomitant pregnancy complications or severe fetal malformations. Each of the perinatal deaths in the current series occurred in pregnancies complicated by compromised maternal condition (preeclampsia or intractable premature labor before 27 weeks' gestation), disturbances of the placental membranes (abruptio placentae or rupture of the amnion), or severe fetal structural abnormalities.

Chescheir and Seeds[2] suggest that the success rate of therapeutic amniocenteses correlates inversely with the gestational age at which the polyhydramnios first manifests itself. Among the 14 affected twins in their series, the four that were first seen after 26 weeks' gestation survived, whereas the 10 that were first seen before 26 weeks died. Bebbington et al.[21] also indicate that a more advanced gestational age at the time of clinical presentation and at the time of delivery correlate with a successful outcome. The data from the current series and from the combined experience in the literature, however, indicate a good survival rate even among fetuses that initially are seen as early as 17 weeks' gestation when managed with serial amniocenteses (see Table III). The median gestational age of initial clinical presentation for survivors managed with serial amniocenteses is approximately 24 weeks in the literature and approximately 20 weeks in the current series.

The potential pregnancy complications associated with serial amniocenteses include abruptio placentae amnionitis, rupture of the membranes, inadvertent puncture of the fetus or umbilical cord, onset of labor, and amniotic fluid embolism. Among the 78 serial amniocenteses for the 21 affected pregnancies described in Table III, procedural complications associated with the amniocenteses are described in four (19%) pregnancies. Two of the pregnancies (9.5%) were noted to be in labor a short time later,[2, 11] but this association is

not necessarily causal inasmuch as preterm labor develops in almost all pregnancies affected by the stuck twin phenomenon. One pregnancy (4.8%) developed amnionitis after an amniocentesis.[2] Vetter and Schneider[10] described inadvertent cordocentesis and therapeutic bloodletting in two cases, but one of these cases underwent 16 other amniocenteses without complication. The relatively high procedural complication rate in the current series (three of eight pregnancies; 37.5%) may reflect the role of ultrasonography in the detection of complications that would otherwise remain undetected.

Potential fetal complications encountered among survivors must be weighed against the strong parental desire for continuation of pregnancy. Prior reports rarely mention complications among survivors of serial amniocenteses in twin pregnancies, but most reports provide only minimal postnatal follow-up data. In the currents series, significant fetal morbidity occurred in two fetuses (12.5%) managed with serial amniocenteses (see Figs. 3 and 4). We postulate that the cerebral infarctions may result from transient cerebral hypoperfusion in a destressed fetus or from embolism via the co-twin. Similar postulates might explain the renal cortical necrosis in one twin. Feingold et al.[11] described a case of renal cortical necrosis in a twin after serial amniocenteses of its polyhydramniotic co-twin, and Dimmick et al.[37] reported renal necrosis and cerebral infarction in a growth-retarded twin with the twin transfusion syndrome.

We conclude that serial amniocenteses may significantly improve the survival rate of twin gestations affected by the stuck twin phenomenon, but that other complications may develop in the survivors. Further studies are required to determine the optimal timing and method of amniocenteses to minimize the fetal and procedural complications.

REFERENCES

1. Mahony BS, Filly RA, Callen PW. Amnionicity and chorionicity in twin pregnancies: prediction using ultrasound. Radiology 1985;155:205-9.
2. Chescheir NC, Seeds JW. Polyhydramnios and oligohydramnios in twin gestations. Obstet Gynecol 1988;71: 882-4.
3. Patten RM, Mack LA, Harvey D, Cyr DR, Pretorius DH. Disparity of amniotic fluid volume and fetal size: problem of the stuck twin—US studies. Radiology 1989;172: 153-7.
4. Arvis P, Roze JM, Priou G, Fraisse E, Grail JY. Acute hydramnios with twin pregnancy. J Gynecol Obst Biol Repr 1983;12:283-6.
5. Achiron R, Rosen N, Zakut H. Pathophysiologic mechanism of hydramnios development in twin transfusion syndrome. J Reprod Med 1987;32:305-8.
6. Pretorius DH, Manchester D, Barkin S, Parker S, Nelson TR. Doppler ultrasound of twin transfusion syndrome. J Ultrasound Med 1988;7:117-24.
7. Weir PE, Ratten GJ, Beischer NA. Acute polyhydramnios—a complication of monozygous twin pregnancy. Br J Obstet Gynaecol 1979;86:849-53.
8. Wittmann BK, Farquharson DF, Thomas WDS, Baldwin VJ, Wadsworth LD. The role of feticide in the management of severe twin transfusion syndrome. Am J Obstet Gynecol 1986;155:1023-6.
9. Benirschke K, Kim CK. Multiple pregnancy. N Engl J Med 1973;288:1276-84.
10. Vetter K, Schneider KTM. Iatrogenous remission of twin transfusion syndrome [Letter]. Am J Obstet Gynecol 1988;158:221.
11. Feingold M, Cetrulo CL, Newton ER, Weiss J, Shakr C, Shmoys S. Serial amniocenteses in the treatment of twin-to-twin transfusion complicated with acute polyhydramnios. Acta Genet Med Gemellol 1986;35:107-13.
12. Rivett LC, Hydramnios. J Obstet Gynaecol Br Emp 1933;40:522-5.
13. Erkskine JP. A case of acute hydramnios successfully treated by abdominal paracentesis. J Obstet Gynaecol Br Emp 1944;51:549-51.
14. Danziger RW. Twin pregnancy with acute hydramnios treated by paracentesis uteri. Br Med J 1948;2:205-6.
15. Brown GR. Acute hydramnios treated by abdominal paracentesis. J Obstet Gynaecol Br Emp 1958;65:61-3.
16. Brandt AJ, Bates JS. Transabdominal amniocentesis in hydramnios. Obstet Gynecol 1961;17:392-4.
17. Brown GR, Macaskill S. Acute hydramnios with twins, successfully treated by abdominal paracentesis. Br Med J 1961;1:1739.
18. Hutfield DC. The successful management of acute hydramnios in a twin pregnancy. Br J Clin Pract 1962; 16:350-2.
19. Brown GR. Acute polyhydramnios—a complication of monozygous twin pregnancy [Letter]. Br J Obstet Gynaecol 1980;87:255.
20. Mills WG. Acute polyhydramnios—a complication of monozygous twin pregnancy [Letter]. Br J Obstet Gynaecol 1980;87:256.
21. Bebbington MW, Wittmann BK. Fetal transfusion syndrome: antenatal factors predicing outcome. Am J Obstet Gynecol 1989;160:913-5.
22. Hill LM. Abnormalities of amniotic fluid. In: Nyberg DA, Mahony BS, Pretorius DH, eds. Diagnostic ultrasound of fetal anomalies. Chicago: Year Book, 1990:38-66.
23. Brown DL, Benson CB, Driscoll SG, Doubilet PM. Twin-twin transfusion syndrome: sonographic findings. Radiology 1989;170:61-3.
24. Nylander PPS. The phenomenon of twinning. In: Barron SL, Thompson AM, eds. Obstetrical epidemiology. San Francisco: Academic Press, 1983:143.
25. Braat DDM, Bernardus RE, Arts NFT, Rajnhere JR. Twin pregnancy: case reports illustrating variations in transfusion syndrome. Eur J Obstet Gynecol Reprod Biol 1985;19:383-90.
26. Johnson SF, Driscoll SG. Twin placentation and its complications. Semin Perinatol 1986;10:9-13.
27. Robertson EG, Neer KJ. Placental injection studies in twin gestation. Am J Obstet Gynecol 1983;147:170-4.
28. Sekiya S, Hafez ESE. Physiomorphology of twin transfusion syndrome: a study of 86 twin gestations. Obstet Gynecol 1977;50:288-92.
29. Giles WB, Trudinger BJ, Cook CM. Fetal umbilical artery flow velocity waveforms in twin pregnancies. Br J Obstet Gynaecol 1985;92:490-7.
30. Giles WB, Trudinger BJ, Cook CM. Doppler sonography findings of the umbilical arteries in eight cases of twins transfusion syndrome [Letter]. J Ultrasound Med 1989; 8:531-2.

31. Danskin FH, Neilson JP. Twin-to-twin transfusion syndrome: what are appropriate diagnostic criteria? Am J Obstet Gynecol 1989;161:365-9.
32. Kirshon B. Fetal urine output in hydramnios. Obstet Gynecol 1989;73:240-2.
33. Nageotte MP, Hurwitz SR, Vaziri ND, Pandian MR. Atriopeptin in the twin transfusion syndrome. Obstet Gynecol 1989;73:867-70.
34. Panos MZ, Nicolaides KH, Anderson JV, Economides DL, Rees L, Williams R. Plasma atrial natriuretic peptide in human fetus: response to intravascular blood transfusion. Am J Obstet Gynecol 1989;161:357-61.
35. Hashimoto B, Callen PW, Filly RA, Laros RK. Ultrasound evaluation of polyhydramnios and twin pregnancy. Am J Obstet Gynecol 1986;154:1069-72.
36. Pretorius DH, Mahony BS. Twin gestations. In: Nyberg DA, Mahony BS, Pretorius DH, eds. Diagnostic ultrasound of fetal anomalies. Chicago: Year Book, 1990:592-622.
37. Dimmick JE, Hardwick DF, Ho Yuen B. A case of renal necrosis and fibrosis in the immediate newborn period: association with the twin-to-twin transfusion syndrome. Am J Dis Child 1971;122:345-7.

Twin-twin transfusion syndrome

Michael A. Urig, MD,[a] William H. Clewell, MD,[b] and John P. Elliott, MD[b]
Phoenix, Arizona

Twin-twin transfusion syndrome associated with acute polyhydramnios in one sac and severe oligohydramnios in the other, which characteristically is diagnosed between 18 and 28 weeks, is associated with a high mortality rate for the involved twins. Patients who are managed without intervention have essentially 100% perinatal mortality. Nineteen patients with this diagnosis were treated at Good Samaritan Medical Center over a 5-year period. Because of the known perinatal mortality and because of early experiences with the twin-twin transfusion syndrome, we began to actively intervene in such patients with various modes of therapy. As experience was gained, it was found that repeated therapeutic amniocenteses, if performed before severe maternal abdominal distention or labor, appears to be beneficial. (Am J Obstet Gynecol 1990;163:1522-6.)

Key words: Twin-twin transfusion, stuck twin, polyhydramnios

Schatz[1] was the first author to describe the phenomenon of shared circulation between monochorionic twins. Anastomoses have been documented between arteries, veins, and between arteries and veins of each twin, but of greatest importance are the arteriovenous anastomoses that proceed through a placental cotyledonary capillary bed and appear to result in the most dramatic circulatory changes in the twins. Schatz reported that this "third circulation" was usually accompanied by a large arteriovenous anastomosis in the opposite direction to compensate for the inequalities that this third circulation provided. This was the explanation for the finding of some normal outcomes in cases of arteriovenous anastomosis. In the situations in which there was no corresponding large-vessel anastomosis in the opposite direction, it was hypothesized that the "third circulation" predominated and resulted in the transfusion syndrome. When the unequal vascular anastomosis is established the donor twin becomes anemic, hypovolemic, oligohydramnic, and as a result, grows slower than the other twin. The recipient twin becomes polycythemic, hypervolemic, and polyhydramnios develops because of increased urine production to relieve the circulatory overload.[2]

From the Department of Obstetrics and Gynecology[a] and the Division of Maternal-Fetal Medicine,[b] Good Samaritan Medical Center.
Received for publication October 27, 1989; revised June 25, 1990; accepted July 27, 1990.
Reprints not available.
6/1/24147

Material and methods

The medical records of Good Samaritan Medical Center were reviewed for the discharge diagnoses of twin pregnancy with associated polyhydramnios. From this group, all 19 patients with twins and associated acute polyhydramnios of one twin and oligohydramnios of the other twin were selected. There was an estimated gestational age difference by ultrasonography of at least 1.5 weeks between the twins in each pair. All diagnoses were made prenatally; the presenting problem was rapid increase in fundal height. In each patient the polyhydramnios was associated with the larger twin, whereas the smaller twin was characteristically "stuck" to the uterine wall, probably as a result of compression from the polyhydramnic twin sac combined with its own oligohydramnios. These characteristics, which are the basis of our diagnosis, are the

same ultrasonographic findings described by other authors.[3-7] These patients represented the worst extreme of the twin-twin transfusion syndrome spectrum with severely discordant growth, acute polyhydramnios, and essentially total oligohydramnios, so that initially the ultrasonographic appearance is of monoamniotic gestation. Patients who did not meet these rigid criteria were not included in this study.

All placentas were found to be monochorionic by gross and microscopic examination. A systemic search for anastomotic vessels was not routinely performed.

Results

Between 1983 and 1987 there were 29,844 deliveries at Good Samaritan Medical Center with 565 sets of twins (incidence of 1:53). There were 19 patients with the diagnosis of twins with acute polyhydramnios and twin-twin transfusion syndrome, an incidence of 1:30 twin pregnancies at our referral center.

The gestational age at the time of diagnosis ranged from 18 to 29 weeks (mean, 22.7 weeks), the majority of pregnancies were in the 21- to 24-week period. The mean gestational age at the time of delivery was 27.3 weeks (excluding elective terminations).

The patients were separated into four groups based on different management philosophy as experience was gained.

Group 1 consisted of five patients early in our experience in whom no intervention was attempted (patients 1 through 5). These patients were seen with the mentioned previously symptoms of rapid uterine distention and preterm labor with cervical change. Historically, twin-twin transfusion syndrome was believed to be associated with essentially 100% mortality at this gestation. Despite tocolysis, all five patients progressed to active labor with rupture of membranes and delivery of nonviable infants (Table I). One patient (no. 4) had intrauterine fetal death of both twins in the course of labor.

Group 2 consisted of patients referred for twin-twin transfusion syndrome in whom preterm labor had already begun. Patients experienced contractions (>two contractions per 10 minutes) and had documented cervical change. Repeated therapeutic amniocenteses were attempted in four patients after they were in preterm labor (patients 6, 7, 8, and 9). It was evident that the removal of amniotic fluid during active labor was not effective in significantly prolonging pregnancy (Table II). Two infants survived (25% survival rate); however these infants were 29 weeks' gestational age at the time of diagnosis.

Group 3 included five patients who initially were seen with severe twin-twin transfusion syndrome with both twins viable, but with a change in intrauterine physiology with the death of one twin (patients 10 through 14). One patient (no. 11) elected termination of the living twin 23 days after the death of the first twin. One patient (no. 14) had the surgical removal of one twin at 21 weeks following three therapeutic amniocenteses.[8] Patient 12 had continued polyhydramnios in the amniotic sac of the recipient twin after death of the donor twin. This patient had therapeutic amniocentesis with removal of 1800 ml of amniotic fluid. The polyhydramnios never reaccumulated and she was delivered at term (Table III). The other three patients did not undergo amniocentesis.

Table I. Cases of twin-twin transfusion syndrome with no intervention

Patient No.	*Gestation at diagnosis (wk)*	*Days gained after diagnosis*	*Survived*
1	21	0	0
2	21	0	0
3	22	0	0
4	24	0	0
5	18	0	0
			0/10 (0%)

The four pregnancies that continued (patients 10, 12, 13, and 14) gained an average of 69 days' gestation after diagnosis. Three of the four remaining infants lived. The remaining twin (patient 14) died after birth during operation to correct congenital aortic stenosis after prolongation of pregnancy from 21 to 27 weeks.

Group 4 comprised five patients from our most recent experience in whom aggressive amniocentesis was begun before any signs of preterm labor (patients 15 through 19). Aggressive amniocentesis was defined as repeated amniocenteses that were performed in an attempt to maintain the level of the amount of fluid surrounding the recipient twin at a level as normal as possible. One to five liters of fluid was removed over a 30-minute to 2-hour period. The amount removed was sufficient to maintain a low-normal to normal amount of amniotic fluid in the recipient's sac. Patients received prophylactic tocolysis with intravenous magnesium sulfate during and after therapeutic amniocentesis. In two patients it was found that initial aggressive removal of fluid early in the disease process appeared to stop the transfusion progression (patients 17 and 19). The average number of days gained was 64.2 days (Table IV). Two infants died from severe prematurity after prolongation of pregnancy from 22 to 25 weeks (patient 15), and two infants died of sepsis after prolongation of pregnancy from 21 to 27 weeks (patient 19). There was an overall survival rate of 60% in this group.

Therapeutic amniocentesis before the onset of preterm labor is significantly more effective than amniocentesis after labor has started in prolonging gestation

Table II. Therapeutic amniocentesis after the onset of preterm labor in twin-twin transfusion syndrome

Patient No.	*Gestation at diagnosis (wk)*	*Days gained*	*No. of amniocenteses*	*Average volume per tap*	*Survived*
6	29	8	2	2400	2
7	21	3	2	900	0
8	26	4	3	1200	0
9	24	3	3	1000	0
					2/8 (25%)

Table III. Death of one twin in twin-twin transfusion syndrome

Patient No.	*Gestation at diagnosis (wk)*	*Days gained after death*	*Survived*
10	24	77	1
11	21	23 (Terminated)	0
12	22	80	1
13	19	74	1
14	21	45	0
			3/10 (30%)

beyond 27 weeks ($p \leq 0.03$) and beyond 32 weeks ($p \leq 0.01$) (χ^2 analysis). Therapeutic amniocentesis is not significantly different in effect than the death of one twin in prolonging gestation beyond 27 weeks or 32 weeks (χ^2 analysis).

Comment

A review of the literature with regard to the twin-twin transfusion syndrome reveals a wide spectrum of patients labeled with this diagnosis. Because of its relatively infrequent presentation, many of the published accounts are single case reports. Some reports indicate a relatively good prognosis for this syndrome.[9-12]

A continuum of dysfunction clearly exists under the label twin-twin transfusion syndrome. Mild manifestations of this process are probably represented by reports of low mortality and morbidity associated with twins delivered with discordant weights and hemoglobin concentrations.[12-14] One such report[14] detailed only seven sets of twins that were premature of 35 cases reported. Rausen et al.[12] reported a significant incidence of term pregnancies, and many did not have associated polyhydramnios. Patients in these reports seem to represent the benign end of the spectrum of twin-twin transfusion syndrome, which manifests itself later in pregnancy and allows delivery at a later gestational age.

Of greater significance are those patients who are seen with acute polyhydramnios at 18 to 28 weeks, when morbidity and mortality approach 100%. Weir et al.[10] reported eight patients with twin-twin transfusion syndrome and acute polyhydramnios treated expectantly with 100% perinatal mortality. Chescheir and Seeds[7] reported five cases, with 100% mortality in true severe twin-twin transfusion syndrome, and four surviving fetuses in two patients in whom the diagnosis was questionable (one set of dichorionic twins, one male and one female), and one set of twins without polyhydramnios or oligohydramnios. Schneider et al.[15] reported a survival rate of 44% after use of amniocentesis to intervene in nine cases of severe twin-twin transfusion syndrome.

These diverse outcome data are consistent with Schatz's original description of the syndrome. He theorized that many arteriovenous anastomoses were accompanied by reciprocal communications that minimize the discrepancy of blood flow. The patients who are delivered at later gestations probably have such compensatory anastomoses. The infants are born with weight and hemoglobin discrepancies but are not commonly complicated by polyhydramnios/oligohydramnios.

Acute polyhydramnios is probably the most impressive finding in patients who are seen with early severe twin-twin transfusion syndrome, and the polyhydramnios is usually the presenting problem. All patients in our series had acute polyhydramnios. The acute polyhydramnios is usually the cause of preterm labor and preterm rupture of membranes that complicate this problem. All patients in this series had a larger twin with polyhydramnios associated with a smaller twin "stuck" to the uterine wall in an oligohydramnic sac. They were at the severe end of the continuum of twin-twin transfusion syndrome; the mortality in such cases is expected to be 100%.

Amniocentesis has been found to be a safe therapeutic method of relieving maternal symptoms as a result of abdominal distention from polyhydramnios.[11, 15-19] Several authors have suggested that pregnancy can be prolonged in the situation of acute polyhydramnios and twin-twin transfusion syndrome.[11, 15-17, 19] Two groups of patients in our series were treated with aggressive amniocentesis (groups 2 and 4). Traditionally, small amounts of fluid (200 to

Table IV. Therapeutic amniocentesis before onset of preterm labor in twin-twin transfusion syndrome

Patient No.	*Gestation at diagnosis (wk)*	*Days gained after diagnosis*	*No. of amniocentesis*	*Average volume per tap (ml)*	*Survived*
15	22	25	7	1360	0
16	21	119	3	1910	2
17	22	80	1	2000	2
18	25	56	7	3075	2
19	21	41	1	1000	0
					6/10 (60%)

500 ml) have been removed during amniocentesis. It is commonly believed that drainage of large amounts of amniotic fluid might cause abruptio placentae or preterm labor. None of the patients in this series from whom large amounts of fluid were removed had evidence of abruptio placentae. In our patients amniocentesis in an actively contracting uterus was not effective in prolonging pregnancy. The only patients who had significant preterm labor during or after amniocentesis were those in group 2 who were already having significant contractions with cervical change at the time of amniocentesis. Caldeyro-Barcia et al.[20] reported that patients at risk for preterm labor who had amniocentesis for polyhydramnios were those with strong uterine contractions at the time of amniocentesis. His review also showed that the incidence of preterm labor in patients with polyhydramnios was much lower if the amniocentesis was performed earlier in pregnancy when uterine contractility is low, and before severe distention. Our experience has been consistent with this finding. If amniocentesis can be performed at the time of diagnosis, and before significant contractions are present, there is a reduced chance of premature labor associated with therapeutic amniocentesis; in this group of five patients (Table IV), 60% survival was noted.

After amniocentesis had been initiated, our goal was to maintain the amniotic fluid volume around both fetuses at a level as close as possible to the normal range. As suggested by Mills,[17] we drained amniotic fluid as often and as rapidly as necessary to maintain the normal amniotic fluid volume in each sac (range, repeat in 1 day to 3 weeks). The largest amount of fluid removed from our group of patients over an 8-week course of therapy was 19 L; 5 L was the largest volume removed at one time. The patients in this series who gained a minimal number of days' gestation were either in preterm labor at the time of the first amniocentesis or were seen with premature rupture of membranes (groups 1 and 2). Chescheir and Seeds[7] did not appreciate significant lengthening of gestation in their three patients who underwent therapeutic amniocentesis. Uterine activity and cervical dilatation were not mentioned and may have been contributing factors. In their series chorioamnionitis developed in one patient after amniocentesis. No patient in our series had signs of chorioamnionitis, and antibiotics were not routinely used prophylactically.

We suggest that amniocentesis be performed for both fetal and maternal reasons. We observed normal fluid volumes around both twins after therapeutic amniocenteses in numerous patients. The only other report of this phenomenon is by Schneider et al.[15] In their study a placental vessel was inadvertently punctured during amniocentesis. Our experience suggests that repeated, aggressive amniocenteses reduce the rate of fluid accumulation in most cases. Only one therapeutic amniocentesis was needed in two cases and in the other three cases the repeated amniocenteses generally occurred after an increasing number of days between amniocenteses. The physiologic reason for this response is uncertain. It is possible that the transfusion syndrome begins at a low level, probably between 16 and 20 weeks. The recipient twin then has an increase in its glomerular filtration as a result of increased renal perfusion. With the accumulation of fluid in the recipient's amniotic sac, the donor twin and its portion of the placenta become compressed and increase the rate of transfusion to the recipient twin. Intravascular hypovolemia in the donor results in decreased renal perfusion and oliguria. It is possible that the relief of pressure exerted by the recipient's polyhydramnios on the donor twin's placental vessels alone is sufficient to partially reverse this phenomenon.

Our experience supports the fact that the intrauterine environment can be normalized if the physiologic characteristics are changed early in the course of this transfusion situation, by therapeutic amniocentesis or the death of one twin. Usually therapeutic amniocentesis should be started in the 16- to 21-week period for best results.

Alternative approaches to the twin-twin transfusion syndrome include fetal surgery or selective in utero termination. These alternatives should be considered only in very severe, early situations, and only after failed attempts at therapeutic amniocentesis.[7, 21] The option of selective in utero termination can lead to

other serious complications involving embolic phenomena and coagulation defects in the remaining twin.[22-24] This is an experimental procedure and is fraught with technical and ethical problems.

The condition of twin-twin transfusion syndrome associated with acute polyhydramnios has a grave prognosis. In most cases, the diagnosis is made at a gestational age when survival for even a normal singleton is doubtful. These fetuses are normal genetically but are subjected to extreme physiologic changes as a result of the abnormal circulatory condition. The goal of therapy should be to prolong the pregnancy to increase the chance of fetal survival.

Of the reports of twin-twin transfusion syndrome in the second trimester when no therapy was instituted, there was essentially 100% perinatal mortality unless one twin died in utero. Twin-twin transfusion syndrome is a rare problem but contributes significantly to perinatal mortality in twins. The survival of such twins can be improved with active early intervention with aggressive therapeutic amniocentesis.

REFERENCES

1. Schatz F. Arch Gynaekol 1875;7:336.
2. Benirschke K, Driscoll S. The pathology of the human placenta. New York: Springer-Verlag, 1967:106-17.
3. Elajalde BR, Elajalde MN, Wagner AM, et al. Diagnosis of twin-twin transfusion syndrome at 18 weeks gestation. JCU 1983;11:442-6.
4. Wittman B, Baldwin V, Nichol B. Antenatal diagnosis of twin transfusion syndrome by ultrasound. Obstet Gynecol 1981;58:123-7.
5. Brennan JN, Diwan RV. Feto-fetal transfusion syndrome: prenatal ultrasound diagnosis. Radiology 1982; 143:535-6.
6. Hashimoto B, Callen PW, Filly RA, et al. Ultrasound evaluation of polyhydramnios and twin pregnancy. Am J Obstet Gynecol 1986;154:1069-72.
7. Chescheir NC, Seeds JW. Polyhydramnios and oligohydramnios in twin gestations. Obstet Gynecol 1987;71: 882-4.
8. Urig MA, Simpson GF, Elliott JP, Clewell WH. Midtrimester surgical removal of one twin to treat twin-twin transfusion syndrome. Fetal Ther 1988;3:185-8.
9. Benirschke K, Kim CK. Multiple pregnancy. I. N Engl J Med 1973;288:1273-84.
10. Weir PE, Ratten G, Beisher N. Acute polyhydramnios—a complication of monozygous twin pregnancy. Br J Obstet Gynaecol 1979;86:849-53.
11. Finegold M, Cetrulo CL, Newton J, et al. Serial amniocenteses in the treatment of twin to twin transfusion complicated with acute polyhydramnios. Acta Genet Med Gemellol (Roma) 1986;35:107-13.
12. Rausen AR, Seki M, Strauss L. Twin transfusion syndrome. J Pediatr 1965;66:613-28.
13. Galea P, Scott JM, Goel KM. Feto-fetal transfusion syndrome. Arch Dis Child 1982;57:781-4.
14. Tan KL, Tan R, Tan SH, et al. The twin transfusion syndrome. Clin Pediatr 1979;18:111-4.
15. Schneider KTM, Vetter K, Huch R. Acute polyhydramnios complicating twin pregnancies. Acta Genet Med Gemellol (Roma) 1985;34:179-84.
16. Brandt AJ, Bates JS. Transabdominal amniocentesis in hydramnios. Obstet Gynecol 1961;17:392-4.
17. Mills WG. Letter to the editor. Br J Obstet Gynaecol 1980;87:255.
18. Brown GR. Letter to the editor. Br J Obstet Gynaecol 1980;87:255.
19. Montan S, Jorgensen C, Sjoberg N. Amniocentesis in the treatment of acute polyhydramnios in twin pregnancies. Acta Obstet Gynecol Scand 1985;64:537-9.
20. Caldeyro-Barcia R, Pose SV, Alvarez H. Uterine contractility in polyhydramnios and the effects of withdrawal of the excess of amniotic fluid. Am J Obstet Gynecol 1957;73:1238-54.
21. Wittman BK, Farquharson DF, Thomas WDS, et al. The role of feticide in the management of severe twin transfusion syndrome. Am J Obstet Gynecol 1986;155: 1023-6.
22. Szymonowicz W, Preston H, Yu VYH. The surviving monozygotic twin. Arch Dis Child 1986;61:454-8.
23. Hanna JH, Hill JM. Single intrauterine fetal demise in multiple gestation. Obstet Gynecol 1984;63:126-30.
24. Hagay ZJ, Mazor M, Leiberman JR. Multiple pregnancy complicated by a single intrauterine fetal death. Obstet Gynecol 1985;66:837-8.

Pregnancy in a patient with treated Wilson's disease: A case report

Patrick Dupont, MD, Olivier Irion, MD, and François Béguin, MD
Geneva, Switzerland

Pregnancy should have a successful outcome in a patient with treated Wilson's disease if complications are excluded before conception. Chelating treatment must be maintained, although there is some concern about its teratogenicity. We describe the course of pregnancy in a patient followed up in our department.
(AM J OBSTET GYNECOL 1990;163:1527-8.)

Key words: Wilson's disease, pregnancy, penicillamine, teratogenicity, trientine

Wilson's disease is an autosomal recessive disorder of copper metabolism characterized by liver cirrhosis and central nervous system dysfunctions. Before the advent of penicillamine, successful pregnancies were rare events. We report one pregnancy followed up in our department and discuss the problems related to this condition and its treatment with regard to pregnancy and the fetus.

Case report

At the age of 21 years, M. K. was admitted to a psychiatric unit for depression. Her history revealed one episode of weakness and abnormal liver function test results at 17 years of age. Two years later she left college because of deteriorating performance. In the following months increasing asthenia, dysarthria, irregular handwriting, difficulty in walking, sialorrhea, and bursts of incoercible laughter developed. Her gynecologic history revealed no menstrual irregularities or miscarriage.

Laboratory tests revealed: thrombocytopenia (78,000/mm^3), slightly elevated liver enzymes, normal serum copper (13 mmol/L), diminished ceruloplasmin (0.13 gm/L, low urine copper excretion (1.1 mmol/day), increased intrahepatic copper concentration (15.8 mmol/gm dry weight), impaired renal functions with glucosuria, phosphaturia, and uricosuria. Wilson's disease was diagnosed. Cerebral computed tomographic scan revealed a discrete bilateral hypodensity affecting the lentiform nuclei and abdominal laparoscopy showed a macronodular liver cirrhosis with hepatosplenomegaly and signs of portal hypertension.

Penicillamine therapy (750 mg/day) and a low copper diet brought rapid clinical improvement. Hepatic function normalized, serum copper level decreased, urinary copper output increased, thrombocytopenia did not improve. Penicillamine was well tolerated and the patient showed no other side effects.

Two years later the patient became pregnant. An abortion was performed.

At age 25 years, when she consulted a new practitioner, the question of motherhood was raised. Data from the medical literature showed that pregnancy in her condition seemed reasonable in an appropriate obstetric setting. Thus she was referred to our department for a thorough evaluation of her condition. Increased urine copper excretion and stable clinical condition proved the patient's compliance to therapy. Liver assessment (ultrasonography, nuclear magnetic resonance, and aminopyrine breathing test) showed hepatosplenomegaly but neither cirrhosis nor hepatic failure. An esophagogastroduodenoscopy revealed nonsymptomatic esophageal varices. With this information pregnancy was deemed reasonable.

Genetic counseling was performed. The risk of a future child inheriting that condition was estimated at 1/200 to 1/400, inasmuch as no other case of Wilson's disease was discovered in the family.

A few months later the woman became pregnant. Penicillamine 750 mg/day was continued. The course of pregnancy was uneventful. Liver function test results were normal and, as expected during pregnancy, serum copper and ceruloplasmin levels increased as did urine copper excretion. Thrombocytopenia remained stable. At the beginning of the third trimester, endoscopy confirmed small esophageal varices. Occult blood tests in stools were negative.

Because of increasing maternal fatigue, birth was induced at 38 weeks' gestation. A girl of 3010 gm was delivered spontaneously. Apgar scores were 9 and 10 at 1 and 5 minutes, respectively, and arterial pH was 7.30. Cord blood showed both normal serum copper (8.3 mmol/L) and ceruloplasmin (0.14 gm/L) in the infant.

The postpartum period was uneventful for both mother and child.

From the Departement of Obstetrics and Gynecology, Hôpital Cantonal Universitaire de Genève.
Received for publication February 13, 1990; revised April 12, 1990; accepted June 2, 1990.
Reprint requests: Patrick Dupont, MD, Departement of Obstetrics and Gynecology, Hôpital Cantonal Universitaire de Genève, Genève 4, Switzerland 1211.
6/1/22873

Comment

As is evident from this case and others,[1] pregnancy should have a successful outcome in patients with Wilson's disease provided that complications such as portal hypertension and impending hepatic failure are excluded before conception. Chelating treatment must be continued throughout pregnancy at the optimum dosage to avoid any severe relapse or damage to the fetus.

The teratogenic effects of penicillamine remain controversial. Dose-related teratogenesis has been proved in rats. Birth defects have been reported in four newborns of mothers with penicillamine-treated Wilson's disease: two infants had transient cutis laxa and one also had micrognathia, low-set ears, inguinal hernias, and joint mobility. Other defects possibly not related to penicillamine were reported in two patients: bilateral club feet, cerebral palsy, blindness, arthrogryposis multiplex, hip dislocation, and a marked hydrocephalus with intraventricular hemorrhage.[2]

A safer alternative to penicillamine could be triethylene tetramine dihydrochloride (trientine), a newer chelating agent with efficiency that equals penicillamine. Even though teratogenicity in the rat was described, its use in pregnancy as reported in a small series[3] showed no birth defects or copper depletion in the fetus.

REFERENCES

1. Scheinberg IH, Sternlieb I. Wilson's disease: major problems in internal medicine, vol 13. Philadelphia and London: WB Saunders, 1984:156-62.
2. Rosa FW. Teratogen update: penicillamine. Teratology 1986;33:127-31.
3. Walshe JM. The management of pregnancy in Wilson's disease treated with trientine. Q J Med 1986;58:81-7.

Nature of gonadotropin-releasing hormone self-priming of luteinizing hormone secretion during the normal menstrual cycle

Michael J. Sollenberger, MD, Elisabeth C. Carlsen, MD, Robert A. Booth, Jr., MS, Michael L. Johnson, PhD, Johannes D. Veldhuis, MD, and William S. Evans, MD
Charlottesville, Virginia

To investigate further the nature of the gonadotropin-releasing hormone self-priming effect on luteinizing hormone release, we administered two submaximal doses of gonadotropin-releasing hormone 2 hours apart to women at three stages of the menstrual cycle and analyzed the resultant luteinizing hormone secretory episodes with deconvolution analysis. When the characteristics of the secretory episodes associated with the second gonadotropin-releasing hormone challenge were compared with those associated with the first, both an enhanced maximal secretory rate and mass of luteinizing hormone secreted was demonstrable at each phase of the cycle. No differences in the luteinizing hormone secretory event half-duration were detected when the responses to the first and second gonadotropin-releasing hormone doses were compared. These data confirm the gonadal hormone milieu–associated self-priming effect of gonadotropin-releasing hormone on luteinizing hormone release and indicate that it is the rate with which luteinizing hormone molecules are discharged from the pituitary gland, rather than the duration of the secretory episode itself, that provides for the self-priming effect. (AM J OBSTET GYNECOL 1990;163:1529-34.)

Key words: GnRH self-priming, deconvolution analysis

The ambient gonadal hormone environment modulates gonadotropin-releasing hormone (GnRH)–stimulated luteinizing hormone (LH) secretion both in vivo[1-5] and in vitro.[6] Moreover, under appropriate conditions in humans,[4, 7] in animal models,[3, 5, 8-11] and in vitro,[3, 6, 12-14] repetitive administration of GnRH results in significant potentiation of LH secretion by the anterior pituitary gland. This phenomenon of enhanced pituitary responsiveness associated with multiple GnRH challenges has been referred to as "self-priming" and may represent a primary mechanism subserving the preovulatory LH surge.[3, 6, 8-10, 13]

From the Departments of Medicine and Pharmacology, Health Sciences Center, and the Biodynamics Institute, University of Virginia.
Supported by National Institutes of Health RCDA grant No. 1K04 HD00711, American Diabetes Association Feasibility Grant, and University of Virginia Computer Services grant to W.S.E.; National Institutes of Health Research Career Development Award 1K04 HD00634 and University of Virginia Computer Services grant to J.D.V.; National Institutes of Health grant R01 AG05977 to W.S.E. and J.D.V.; United States Public Health Service General Clinical Research grant RR-847; and Diabetes and Endocrinology Research Center grant No. DK 38942.
Presented in part at the Seventieth Annual Meeting of the Endocrine Society, New Orleans, Louisiana, 1988.
Received for publication April 26, 1990; revised July 16, 1990; accepted July 20, 1990.
Reprint requests: William S. Evans, MD, Box 511, Department of Medicine, University of Virginia Health Sciences Center, Charlottesville, VA 22908.
6/1/24038

Despite intensive investigation, the mechanisms that subserve GnRH self-priming remain to be clarified. To date, studies in the human have focused entirely on changes in the circulating concentration of LH after the administration of serial pulses of GnRH. Although of obvious importance with regard to defining the event, these observations have been primarily descriptive in nature and have not provided insight into the determinants of the GnRH-stimulated LH secretory burst. Recently a novel analytic approach known as multiple-parameter deconvolution has been successfully applied to a variety of hormonal secretory profiles.[15] This technique allows for the simultaneous resolution of specific, quantitative features of hormone secretion together with subject-specific metabolic clearance rates. We have used this model in this study to assess LH secretion in response to two submaximal bolus infusions of GnRH administered 2 hours apart in 12 women at three stages of the menstrual cycle. With this design, both the effect of the gonadal hormone milieu on GnRH-stimulated LH secretion (in response to the first bolus of GnRH) and on GnRH self-priming (LH secretion in response to the second versus the first GnRH bolus) could be assessed. Specifically, we tested the hypotheses that the self-priming phenomenon defined previously by changes in serum concentrations of LH may reflect either (1) alterations in one or more attributes encoding for the luteinizing hor-

Table I. Mean (± SEM) serum concentrations of gonadal steroid hormones in normal women at three phases of menstrual cycle*

Menstrual cycle phase	*17β-Estradiol (pg/ml)*	*Progesterone (ng/dl)*
Early follicular	24 ± 7[a]	34 ± 8[a]
Late follicular	119 ± 13[b]	27 ± 4[a]
Midluteal	128 ± 21[b]	1503 ± 284[b]

Entries within each column identified by different superscripts (*a* and *b*) differ significantly (Duncan's multiple-range test, $p < 0.05$).

*n = 12 women for each study phase.

mone secretory events stimulated by GnRH or (2) alterations in the half-life of secreted hormone.

Material and methods

Subjects and experimental procedures. Twenty-nine young women (mean age, 28 years; range, 18 to 40 years) were studied on one or more occasions at the University of Virginia Clinical Research Center. The study was approved by the Human Investigation Committee of the University of Virginia and each subject provided written consent. All volunteers had a normal history and physical examination and normal serum concentrations of LH, follicle-stimulating hormone, prolactin, thyroxine, thyrotropin, and total testosterone. During the month of study the phase of the menstrual cycle was documented by daily blood samples for measurement of 17β-estradiol, progesterone, and LH. Twelve women were studied in each of three stages of the menstrual cycle including the early follicular phase (days 2 to 4 after beginning of the menses), the late follicular phase (1 to 4 days before the LH surge), and the midluteal phase (5 to 9 days after the LH surge). On the day of study blood samples were obtained from an indwelling heparin cannula at 10-minute intervals from 8 AM to 12 noon. GnRH (10 μg intravenously) was given at 8 AM and 10 AM.

To be included in the analysis, the LH concentration response to the GnRH bolus had to be sufficient to fulfill the following criteria: (1) a peak increase of at least 2 mIU/ml above baseline, (2) a peak response occurring within 20 minutes of GnRH injection, (3) a nonzero decay slope, and (4) 95% of samples available for analysis.

Assays. Serum concentrations of prolactin, thyroxine, thyrotropin, testosterone, estradiol, and progesterone were determined by radioimmunoassay. Progesterone radioimmunoassay was performed with reagents supplied by Diagnostic Products (Los Angeles). Serum LH and follicle-stimulating hormone concentrations were measured with reagents from Clinetics Corp. (Tusten, Calif.). Median intrassay coefficients of variation (calculated from each subject's 145 samples collected over the preceding 24 hours) ranged from 4.6% to 6.2%. All samples from an individual woman were analyzed in duplicate in the same assay.

Deconvolution analysis. Serum hormone concentrations result from the combination of the underlying pituitary secretory events and endogenous (subject-specific) metabolic clearance. Deconvolution analysis allows for the simultaneous determination of the quantitative properties of underlying secretory bursts (characterized by a finite mass, amplitude, and duration) and endogenous clearance kinetics.[15] Endogenous clearance kinetics are related to half-lives by the relationship $MCR = (\ln 2/t_{1/2})/V_d$, where MCR is the metabolic clearance rate and V_d represents the volume of distribution. An LH secretory event is represented algebraically by a gaussian distribution of instantaneous molecular secretory rates that are centered around a particular point in time and dispersed with a finite standard deviation. Multiple-parameter deconvolution procedures were applied separately to the LH data obtained after the administration of each GnRH bolus (8 AM to 10 AM and 10 AM to 12 noon).

Statistical analysis. One-way analysis of variance with Duncan's multiple-range test was used to compare interphase differences in serum concentrations of gonadal steroids. Similar analyses of interphase secretory burst properties and LH half-lives were performed after logarithmic transformation of the data because of departures from normality. Comparisons of these parameters for each GnRH bolus were made by nonparametric methods (Wilcoxon signed-rank test). Correlations between two measures were sought by linear regression analysis of untransformed data. Multiple linear regression using the R^2 maximal improvement criterion was applied to define relationships among three or more variables.

Results

Circulating concentrations of gonadal hormones. The mean serum concentrations of gonadal hormones are summarized in Table I. Serum 17β-estradiol concentrations were similar in the late follicular and midluteal phases and significantly higher than in the early follicular phase. Circulating progesterone concentrations were increased in the midluteal phase, but was indistinguishable in the two follicular phases studied.

Analysis of luteinizing hormone secretion in response to the initial GnRH bolus (GnRH 1). Fig. 1 shows GnRH-stimulated LH concentration time series and the corresponding deconvolution-resolved LH secretory impulses obtained in a representative woman studied at three phases of the menstrual cycle. Visual inspection of these data suggested the presence of cycle-dependent differences in LH secretory event amplitude

Table II. Mean (± SEM) LH secretory burst characteristics during early follicular, late follicular, and midluteal phases of menstrual cycle

	LH secretory burst							
	Maximal secretory rate (mIU/ml/min)		*Half-duration (min)*		*Mass (mIU/ml)*		*Half-life (min)*	
Phase	*GnRH 1*	*GnRH 2*	*GnRH 1*	*GnRH 2*	*GnRH 1*	*GnRH 2*	*GnRH 1*	*GnRH 2*
Early follicular	1.5 ± 0.3[a]	2.5 ± 0.5*	8.5 ± 1.5[a]	6.8 ± 0.8	13 ± 4[a]	16 ± 3†	125 ± 14[a]	117 ± 10
Late follicular	3.8 ± 1.4[a]	9.2 ± 2.7†	11.8 ± 1.0[b]	13.9 ± 1.5	39 ± 9[b]	111 ± 26*	117 ± 12[a]	82 ± 8*
Midluteal	6.0 ± 1.3[b]	12.0 ± 1.9†	8.5 ± 1.3[a]	6.4 ± 0.6	48 ± 9[b]	78 ± 11*	64 ± 4[b]	61 ± 4

GnRH (10 μg intravenously) was given at 0 (GnRH 1) and 2 (GnRH 2) hours.

Duration (in minutes) of the computer-resolved LH secretory burst at half-maximal amplitude.

The volume term (milliliters) represents unit distribution volume. Amplitude is the maximal rate of LH secretion attained in the deconvolution-resolved LH secretory burst.

Entries within each GnRH 1 column identified by different superscripts (*a* and *b*) differ significantly (Duncan's multiple-range test of log transformed values, $p < 0.05$).

*Comparisons of secretory burst properties and LH half-lives (GnRH 1 vs GnRH 2) were made by nonparametric options (Wilcoxon signed-rank test): $p < 0.01$.

†Comparisons of secretory burst properties and LH half-lives (GnRH 1 vs GnRH 2) were made by nonparametric options (Wilcoxon signed-rank test): $0.01 < p < 0.05$.

(maximal secretory rate) and half-duration in response to the first and second challenges with GnRH. Analysis of the deconvolution-resolved secretory event characteristics confirmed this visual impression. Table II summarizes the LH secretory event characteristics inferred in response to the first bolus of GnRH as a function of the phase of the menstrual cycle. Although there was a trend toward a higher maximal LH secretory rate in response to GnRH during the late follicular versus the early follicular phase, a significant difference was not realized ($p = 0.076$). In contrast, the maximal secretory rate during the midluteal phase was significantly greater than that observed in the early follicular ($p = 0.0002$) or late follicular ($p = 0.021$) phases. However, during the late follicular phase the duration of the GnRH-associated LH secretory event was substantially longer than that documented during the early follicular ($p = 0.022$) and midluteal ($p = 0.041$) phases. The mass of LH released in response to GnRH was greater in both the late follicular and midluteal phases of the cycle compared with the early follicular phase ($p = 0.002$ and 0.0001, respectively), but the former two were indistinguishable ($p = 0.376$). Whereas the half-life estimates of LH released during the early follicular and late follicular phases were similar ($p = 0.754$), that of the material secreted in the midluteal phase was significantly shorter ($p = 0.0001$).

Comparison of luteinizing hormone secretory response to GnRH boluses (GnRH 1 vs GnRH 2). Table II also shows the LH secretory event characteristics in response to a second challenge with a submaximal dose of GnRH administered 2 hours after the initial bolus. During the early follicular phase both the maximal rate of LH secretion and the mass of LH released were greater ($p < 0.01$ and $p < 0.05$, respectively) in response to the second versus the first GnRH challenge. However, neither the secretory event duration nor the half-life of hormone secreted differed. The same pattern was evident during the midluteal phase [i.e., a higher maximal secretory rate ($p < 0.05$) and mass ($p < 0.01$)], but indistinguishable duration and half-life of LH were found when the response to the second GnRH bolus was compared with the response to the first. During the late follicular phase, both the maximal secretory rate and mass of hormone secreted were increased in response to the second challenge with GnRH ($p < 0.05$ and $p < 0.01$, respectively). However, during the late follicular phase, and in contrast to the results obtained in the early follicular and midluteal phases, the half-life of secreted LH in response to the second injection of GnRH was significantly ($p < 0.01$) shorter than that released in response to the first dose. No difference in the secretory burst duration was detected.

Correlations among GnRH-stimulated secretory event characteristics and the circulating concentrations of the gonadal hormones. When all women were considered as a group across various stages of the menstrual cycle, multiple stepwise linear regression analysis demonstrated that serum estradiol concentrations were significantly predictive of the mass of LH secreted after the first GnRH injection ($p < 0.0001$ and $R^2 = 0.41$ for mass). Serum estradiol levels also predicted the maximal rate of LH release after the first GnRH pulse ($p = 0.004$). Serum progesterone concentrations predicted the LH half-life ($p < 0.004$) and the half-duration ($p < 0.02$) of the first LH secretory burst.

When individual phases of the menstrual cycle were considered alone, the following findings emerged: (1)

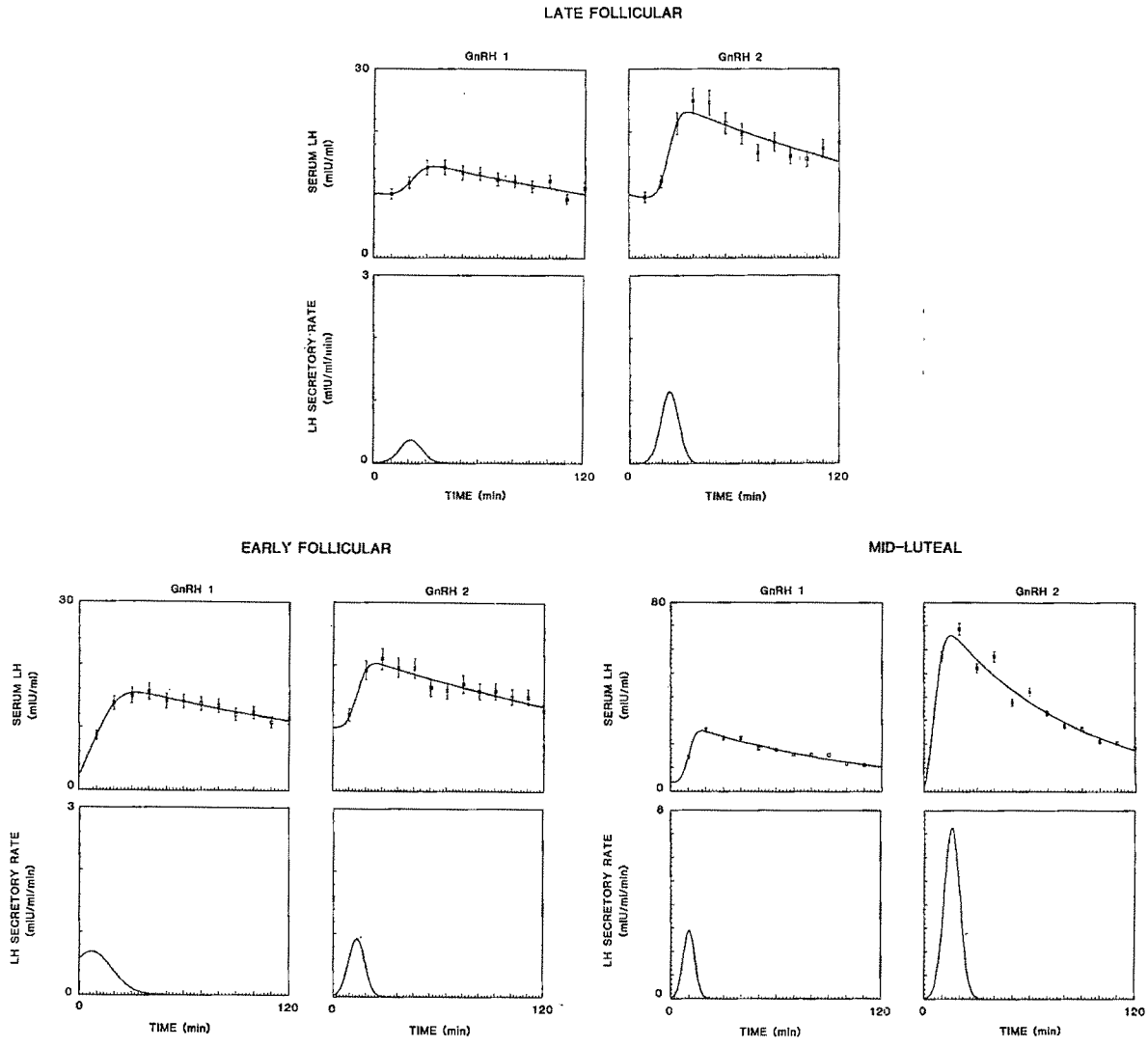


Fig. 1. Illustrative profiles of the deconvolved LH secretory impulses in response to two GnRH challenges (*GnRH 1* and *GnRH 2*) in a representative normal woman. For each phase of the menstrual cycle the *upper panel* depicts measured serum LH concentrations (mean ± SD) and the fitted reconvolution curve *(continuous line)*, which is based on the calculated secretion and clearance parameters. The resolved LH secretory impulses are shown in the *lower panel.*

in the early follicular phase, serum estradiol levels were predictive of both the second luteinizing hormone secretory burst's mass (or its maximal rate) and its half-duration ($p < 0.05, R^2 \geq 0.48$); (2) in the early follicular (but not late follicular) phase, serum progesterone concentrations predicted LH secretory burst mass after the first pulse of GnRH ($p = 0.03$, $R^2 = 0.42$); (3) in the late follicular and midluteal phases, serum estradiol values correlated with the mass and the maximal rate of luteinizing hormone secreted after the first pulse of GnRH ($p < 0.03, R^2 \geq 0.48$); (4) in the midluteal phase, serum 17β-estradiol predicted both the mass of LH secreted and the half-duration of the second LH release episode ($p < 0.03$, $R^2 > 0.63$); and (5) in the midluteal phase, serum progesterone concentrations were significantly predictive of the mass of LH secreted after the second GnRH pulse ($p = 0.008$, $R^2 = 0.56$). Thus, in each of the different phases of the menstrual cycle, serum estradiol concentrations correlated positively and significantly with the mass and maximal rate of LH secretion attained in response to exogenous pulses of GnRH.

Comment

Although the importance of the preovulatory LH surge within the context of normal reproductive function has long been recognized, the cellular events that signal the dramatically increased serum concentrations of LH at midcycle remain incompletely defined. Data obtained in animal models suggest that the secretion of

GnRH into the hypothalamic-hypophysial portal circulation is markedly enhanced coincident with the preovulatory LH surge.[16] However, it is generally accepted that increased GnRH secretion does not solely account for the midcycle LH surge; rather it is thought that the surge reflects a situation in which increased concentrations of GnRH impinge on an anterior pituitary gland that has been rendered considerably more responsive to the releasing hormone just at midcycle. Accordingly, significant investigative interest has focused on the mechanisms subserving this alteration in gonadotroph responsivity.

That GnRH-stimulated LH release is dependent on the gonadal hormone milieu has been documented in a variety of in vivo and in vitro studies.[1-6] Of particular interest are the reports suggesting that, under appropriate conditions, enhanced LH release is observed in response to GnRH after an initial challenge with a "priming" dose of the hypothalamic peptide.[3-6, 7-14] Although this phenomenon appears to be dependent on the gonadal hormone environment,[2, 11-14] the mechanisms involved remain poorly understood. In this study we coupled a moderately intensive sampling paradigm with analysis of the resultant LH concentration time series by use of a multiple-parameter deconvolution procedure to appraise in some detail the determinants of the GnRH secretory-priming event at several stages of the menstrual cycle.

Our current results are consistent with previous studies in women that have documented that the stage of the menstrual cycle,[2] presumably reflecting the gonadal hormone environment,[1] influences the serum concentrations of luteinizing hormone achieved in response to a single bolus of GnRH. The use of deconvolution precedures to "unravel" the GnRH-associated secretory event now provides an enhanced understanding of how this menstrual state-specific modulation occurs. Thus the maximal rate of LH secretion (as distinct from serum LH concentrations per se) achieved in response to GnRH in the midluteal phase is significantly greater than that observed in the early or late follicular phases. In contrast, the duration of the secretory episode in the late follicular phase is prolonged in comparison with the early follicular and midluteal phases. It should be noted that the duration of secretion is considerably shorter than that of the serum concentration peak. Thus the enhanced maximal secretory rate in the midluteal phase and the longer secretory event duration in the late follicular phase are responsible for the greater mass of hormone secreted in these two phases when compared with that secreted in the early follicular phase. Such information about secretion requires a technique such as deconvolution analysis, and cannot be inferred from simple inspection of the serum LH concentration peak.[15] Clearly, these studies do not address the question of which particular intracellular processes subserve the menstrual stage-associated alterations in maximal LH secretory rate and secretory event duration. However, in consideration of this experimental design in which the hypothalamic peptide was administered exogenously, these data do demonstrate that short-term variations in the amount and/or duration of the GnRH stimulus per se are not required to effect variations in the characteristics of the LH secretory event. More prolonged variations in the characteristics of the endogenous GnRH pulse signal throughout the menstrual cycle may act to influence pituitary responsiveness.

Our data also support earlier studies that demonstrated GnRH self-priming in the late follicular-midluteal[2] and midfollicular-midluteal[7] phases of the menstrual cycle. However, our results also provide evidence for a previously undescribed potentiating effect of GnRH on certain secretory event characteristics during the early follicular phase. Specifically, the maximal rate of LH secretion achieved in response to the second bolus of GnRH compared with the first was also enhanced in the early follicular phase and the midluteal phase. However, the duration of the secretory events in response to the two consecutive GnRH challenges did not differ. These results imply that it is the rate with which LH molecules are discharged from the pituitary gland within a given amount of time, rather than the duration of release itself, that results in the observed augmentation of LH secretion with repetitive GnRH administration.

Serum estradiol concentrations were significantly predictive of the mass (as well as maximal rate) of LH secreted after the first GnRH injection in the late follicular and midluteal phases. This finding is consistent with an earlier inference by Lasley et al.[1] that estrogen participates in increasing intrapituitary LH stores that are available for release in response to secretagogue action.[1] Of additional interest is the finding that serum estrogen levels also correlated with the mass of LH released after the second pulse of GnRH in the early follicular phase. This observation suggests that both estrogen and prior exogenous GnRH exposure are required in the early follicular phase to demonstrate an increased availability of LH for release. Serum progesterone concentrations were negatively correlated with LH half-life and secretory burst duration, possibly indicating changes in the posttranslational carbohydrate modification of LH molecules with associated changes in the metabolic clearance rate of LH[17] and alterations in the kinetics of GnRH action and/or gonadotroph secretory responses, which result in a decreased secretory burst duration. In the midluteal phase, progesterone concentrations were related to the mass of LH released after the second GnRH pulse. Thus progesterone also may influence the self-priming actions of GnRH.

The half-lives reported for endogenous LH correspond to half-lives of LH (calculated from stable metabolic clearance rates) of 123 minutes,[18] 136 minutes,[19] and 112 minutes,[20] assuming a 3.5 L distribution volume for LH. Thus our LH half-life values of 60 to 120 minutes agree with independently estimated values obtained by steady-state infusion of various exogenous LH preparations. It should be noted that the deconvolution model used in this study provides estimates of LH half-life under steady-state conditions (or approximately its slower second component rate constant in nonequilibrium studies) and does not reflect acute distribution coefficients (initial equilibration half-times after bolus injection), which could be calculated with even more rapid sampling. This observation presumably reflects the fact that LH distribution is virtually complete between consecutive (10-minute) sample observations. Moreover, our results do not disagree with those of Wehmann et al.,[18] who showed a moderate increase in metabolic clearance rate (or decrease in half-life) in the luteal phase.

We thank Sandra Jackson and the nursing staff at the Clinical Research Center for their expert patient care and Catherine Kern and Ginger Bauer for technical assistance with the assays.

REFERENCES

1. Lasley BL, Wang CF, Yen SSC. The effects of estrogen and progesterone on the functional capacity of the gonadotrophs. J Clin Endocrinol metab 1975;41:820-6.
2. Wang CF, Lasley BL, Lein A, Yen SSC. The functional changes of the pituitary gonadotrophs during the menstrual cycle. J Clin Endocrinol Metab 1976;42:718-28.
3. Pickering AJMC, Fink G. Priming effect of luteinizing hormone releasing factor: in vitro and in vivo evidence consistent with its dependence upon protein and RNA synthesis. J Endocrinol 1976;69:373-9.
4. Hoff JD, Lasley CL, Wang CF, Yen SSC. The two pools of pituitary gonadotropin: regulation during the menstrual cycle. J Clin Endocrinol Metab 1977;44:302-12.
5. Higuchi T, Kawakami M. Luteinizing hormone responses to repeated injections of luteinizing hormone-releasing hormone in the rate during the oestrous cycle and after ovariectomy with and without oestrogen treatment. J Endocrinol 1982;93:161-8.
6. Waring DW, Turgeon JL. Luteinizing hormone-releasing hormone-induced luteinizing hormone secretion in vitro: cyclic changes in responsiveness and self-priming. Endocrinology 1980;106:1430-6.
7. Rommler A. Short-term regulation of LH and FSH secretion in cyclic women I. Altered pituitary response to a second of two LH-RH injections at short intervals. Acta Endocrinol [Copenh] 1978;87:248-58.
8. Aiyer MS, Chiappa SA, Fink G. A priming effect of luteinizing hormone-releasing factor on the anterior pituitary gland in the female rat. J Endocrinol 1974;62:573-88.
9. Castro-Vaszquez A, McCann SM. Cyclic variations in the increased responsiveness of the pituitary to luteinizing hormone-releasing hormone (LHRH) induced by LHRH. Endocrinology 1975;97:13-8.
10. Fink G, Chiappa SA, Aujer MS. Priming effect of luteinizing hormone-releasing factor elicited by preoptic stimulation and by intravenous infusion and multiple injections of the synthetic decapeptide. J Endocrinol 1976; 69:359-72.
11. Nazian SJ. Androgenic and estrogenic control of the self-priming effect of LHRH in the castrated male rat. Neuroendocrinology 1986;42:112-9.
12. Beck LV, Bay M, Smith AF, King D, Long R. Steroid priming of the luteinizing hormone response to luteinizing hormone-releasing hormone. J Endocrinol 1978; 77:293-9.
13. Speight A, Fink G. Comparison of steroid and LH-RH effects on the responsiveness of hemipituitary glands and dispersed pituitary cells. Mol Cell Endocrinol 1981;24: 267-81.
14. Padmanabhan V, Leung K, Convey EM. Ovarian steroids modulate the self-priming effect of luteinizing hormone-releasing hormone on bovine pituitary cells in vitro. Endocrinology 1982;110:717-21.
15. Veldhuis JD, Carlson ML, Johnson ML. The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. Proc Natl Acad Sci USA 1987;84:7686-90.
16. Sarkar DK, Chiappa SA, Fink G, Sherwood N. Gonadotropin-releasing hormone surge in pro-oestrous rats. Nature 1976;264:461-3.
17. Dufau ML, Veldhuis JD. Pathophysiological relationships between the biological and immunological activities of luteinizing hormone. In: Burger HG, ed. Balliere's clinical endocrinology and metabolism. Philadelphia: WB Saunders, 1987 vol 1:153-76.
18. Wehmann RE, Blackman MR, Harman SM. Metabolic clearance rates of luteinizing hormone in women during different phases of the menstrual cycle and while taking an oral contraceptive. J Clin Endocrinol Metab 1982; 55:654-9.
19. Marshall JC, Anderson DC, Fraser TR, Harsoulis P. Human luteinizing hormone in man: studies of metabolism and biological action. J Endocrinol 1973;56:431-9.
20. Pepperell RJ, DeKretser DM, Burger HG. Studies on the metabolic clearance rate and production rate of human luteinizing hormone and on the initial half-time of its subunits in man. J Clin Invest 1975;56:118-26.

A complete list of references is available from the authors on request.

The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening

Katherine M. Gillogley, MD,[a] Arthur T. Evans, MD,[a] Robin L. Hansen, MD,[b] Steven J. Samuels, PhD,[a] and K.K. Batra, PhD[c]
Sacramento, California

Universal urine testing for cocaine, amphetamines, and opiates was performed on 1643 women admitted to an obstetric service for a 1-year period with 20.5% having positive results. There were 299 patients with positive toxicology results matched for race and discharge date with patients having negative toxicology and drug history. Significant differences in age, prior obstetric history, prenatal care, alcohol history, and smoking were noted between groups. There was a significant decrease in birth weight, head circumference, length, and gestational age for the drug-positive group, which was most marked in cocaine and multiple drug users. These differences persisted after we controlled for smoking, prenatal care, and prior preterm births. Differences in birth weight and head circumference remained after we controlled for gestational age. Rates of congenital anomalies and abruptio placental were similar between groups. Perinatal substance abuse is independently associated with growth retardation and prematurity. Multiple risk factors are frequently present, necessitating a comprehensive approach to prenatal care. (AM J OBSTET GYNECOL 1990;163:1535-42.)

Key words: Perinatal substance abuse, cocaine, amphetamines, opiates, low birth weight

Perinatal chemical use appears to be increasing dramatically throughout the United States. Obstetric care providers are being confronted with increasing numbers of women who are using chemical substances during pregnancy. Pediatricians are also encountering increasing numbers of newborn infants with evidence of intrauterine exposure to maternal chemical use.

There are many forms of perinatal chemical use. Improper or abusive use of legal chemicals such as alcohol and cigarettes is common and legally tolerated. The misuse of controlled chemicals such as sedatives, cocaine, amphetamines, marijuana, and narcotics is frequently encountered and appears to be increasing. The use of illegal chemicals (those with no medically sanctioned uses) such as crack cocaine, crystal amphetamine, PCP, and LSD has been less apparent during pregnancy until the past decade.

The complications that have been reported with the perinatal use of these chemicals include: low birth weight, prematurity, stillbirth, abruptio placentae, fetal distress, cerebral infarctions, congenital malformations, neonatal toxicity and withdrawal, sudden infant death syndrome, and neurobehavioral abnormalities.[1-6] However, these complications are not limited to or specific for chemical use. There are numerous confounding factors such as smoking, alcohol use, limited or no prenatal care, low socioeconomic status, or poor reproductive history present in many of the pregnancies that involve chemical use. These confounding factors are recognized to be associated with many of the same complications that have been reported with chemical use alone. Previous studies of perinatal chemical use have been limited in their ability to evaluate the contribution of confounding factors to adverse perinatal outcome. Limited patient numbers and inability to identify the chemical use status of all patients in the study population have interfered with analysis of the impact of confounding factors.

The accurate assessment of the extent of perinatal chemical use, its impact on perinatal outcomes, and the interplay with confounding factors requires population-based data. Without such information, appropriate decisions cannot be made regarding allocation of resources and areas of emphasis for patient identification, intervention, and follow-up programs.

This study investigates chemical use in an obstetric population based in an urban perinatal center. All patients underwent urine testing for cocaine, amphetamines, and opiates at the time of obstetric admission.

From the Department of Obstetrics and Gynecology,[a] the Department of Pediatrics,[b] and the Department of Pathology,[c] University of California, Davis.
Received for publication March 27, 1990; revised June 25, 1990; accepted July 27, 1990.
Reprints not available.
6/1/24111

Differences in demographic and historical factors, perinatal outcome, and the effect of confounding factors are analyzed.

Methods

The Perinatal Unit of the University of California, Davis, Medical Center (UCDMC) is a tertiary obstetric center providing primary services in the Sacramento area, as well as referral services for northern California. The obstetric population at UCDMC is primarily urban; 93% have Medi-Cal (Medicaid) or no insurance coverage. Transfers from other hospitals for acute perinatal complications account for approximately 10% of patients. The patients are ethnically diverse; 46% are white, 21.8% Hispanic, 21.7% black, 5% Asian, and 5.5% various other groups.

Since November 1987 all women admitted to the Obstetrics Service at UCDMC have been routinely tested by a urine toxicology screen, which included assays for cocaine, amphetamines, and opiates. Newborn infants admitted to the nurseries at UCDMC are similarly tested if the mother's toxicology screen is positive or if the infant demonstrates evidence of chemical effects or withdrawal. The results are used to provide appropriate medical and social services. Mother-infant pairs testing positive for the above receive a social services evaluation. A Child Protective Services evaluation is performed as deemed necessary by social services or the pediatrician. Results are not reported to criminal authorities.

All toxicology screens on mothers and infants are performed by the UCDMC Toxicology and Drug Analysis Laboratory. Initial testing for cocaine, amphetamines, and opiates is performed by homogeneous enzyme immunoassay technique (EMIT d.a.u.; Syva Company, Palo Alto). The specificity for the major metabolite of cocaine, benzoylecgonine, is 99% at concentrations of 0.3 μg/ml. The detection of amphetamines and methamphetamines is confirmed by gas chromatography equipped with a nitrogen-phosphorus detector. The EMIT assay is quite specific for opiates but does not distinguish between morphine and other opiates. Cases were excluded if the patient tested positive for opiates only and had received any opiate-containing medications before collection of the urine sample.

Detailed patient information was retrospectively obtained through chart review and from computerized discharge data. Between Dec. 1, 1987, and Nov. 30, 1988, 2028 patients were discharged from the UCDMC Obstetrics Service. Of these, 1643 (81.1%) had a documented result available from urine testing for cocaine, amphetamines, and opiates. Cases were excluded if the patient was discharged antenatally and delivered elsewhere.

The control group was selected from women with both a negative toxicology screen and drug history who were delivered at UCDMC during the time of the study. They were matched with toxicology-positive patients for race and nearest discharge date. The drug history was taken from the patient's prenatal chart and on admission by the admitting physician. If a history of current or past use of opiates, cocaine, amphetamines, marijuana, or other illicit chemicals was noted, the patient was excluded from the control group and another patient was selected.

Statistical analysis of continuous variables was performed with Student's t test with analysis of variance and Bonferroni's t test for multiple comparisons. For categorical variables, the χ^2 test or Fisher's exact test was used with the Bonferroni correction for multiple comparisons. Case and control Apgar scores were compared by the Wilcoxon two-sample rank sum test specialized to grouped data. Multiple regression with analysis of covariance was used to control for confounding factors.

Results

A total of 379 patients had positive urine toxicology screens out of 2028 total obstetric admissions and 1643 patients tested with known laboratory results. Charts were available for review on 348 of the toxicology-positive patients. Forty patients were subsequently excluded because they had received opiate-containing medication before collection of the urine sample, which was positive only for opiates. An additional patient was excluded because of suspected mislabeling of the initial positive specimen with a negative repeat screen. Thus there were 338 (20.5%) patients with positive urine toxicology screens out of 1643 screened admissions and after the above exclusions.

Charts were available for review for 307 patients with "true-positive" results. Eight patients were excluded from obstetric and neonatal outcome analysis because they were discharged antenatally and delivered elsewhere. Of the remaining 299 patients, 12 were discharged undelivered and returned to be delivered on a subsequent admission. Six of these had negative toxicology screens on the subsequent delivery admission. These 12 patients were included in the statistical analysis.

The control group consisted of 293 patients with both negative toxicology screens and drug histories, matched with the toxicology-positive patients for race and nearest discharge date. Ninety-eight patients with negative toxicology screens gave a history of prior drug use and were therefore excluded during the control selection process. Three potential control group patients with negative toxicology screens were excluded because their infants had positive toxicology screens, two for cocaine and one for amphetamines.

Table I. Maternal age and obstetric history

	Controls	*Total cases*	*Cocaine*	*Amphetamine*	*Opiates*	*Multichemicals*
Age (yr) (n = 591)	23.5	25.7*	25.8*	25.2†	25.5	27.1*
Gravidity (n = 586)	2.8	4.2*	4.3*	3.9*	4.2†	5.1*
Term (n = 586)	1.1	1.8*	1.8*	1.7*	1.4	1.9*
Preterm (n = 586)	0.11	0.28*	0.26†	0.23	0.42†	0.40*
Spontaneous abortion (n = 586)	0.30	0.42†	0.41	0.41	0.58	0.40
Induced abortion (n = 586)	0.39	0.78*	0.78*	0.60	0.79	1.29*
Living (n = 586)	1.1	1.9*	1.9*	1.7*	1.6	2.2*

Values are means.
*$p < 0.01$, comparison with controls.
†$p < 0.05$, comparison with controls.

Toxicology results. Cocaine alone was the most common cause of a positive toxicology screen, being detected in 139 patients (46% of positives). Amphetamine-methamphetamine alone was found in 106 patients (35%). More than one of these chemicals were detected in 35 patients (12%), while only 19 patients were found to be positive for opiates alone.

Age. The mean age was 25.7 years for study patients versus 23.5 years for controls ($p < 0.001$) (Table I). It is of interest that the users of multiple drugs had the highest mean age at 27.1 years.

Obstetric history. There were significant differences ($p < 0.05$) between study and control patients for gravidity, preterm births, abortions (both induced and spontaneous), and number of living children (Table I). The difference in gravidity persisted even after control for differences in maternal age ($p < 0.001$). When primigravid women were excluded, there were no significant differences between the groups for the proportion of preterm births or spontaneous or induced abortions to prior pregnancies. There was a trend that did not reach statistical significance ($p = 0.13$) for an increased proportion of preterm births in the drug-positive group.

Race. Study patients and controls were matched for race. The toxicology-positive patient group comprised 44% blacks, 42% whites, 10% Hispanics, and the remainder either "other" or unknown ethnic definitions. This represents a significant disparity from the racial distribution of the total UCDMC obstetric population, which consists of 22% blacks, 46% whites, 22% Hispanics, and 5% Asians. There were significant racial differences between the users of the different types of chemicals (Fig. 1)

Marital status. Marital status was assigned as single, married, separated, or divorced. There was a difference approaching significance ($p = 0.05$) between the study patients and controls with these categories. There was a significant difference between cases and controls ($p < 0.02$) in married versus unmarried status. Cocaine and multiple chemical users were least likely to be married.

Prenatal care. The majority of patients studied received their prenatal care in the UCDMC Obstetrics Clinic (53.2% of study patients and 73.3% of controls). There was a highly significant difference ($p < 0.001$) between the two groups in failure to obtain prenatal care (Table II). The group of opiates-only patients received the greatest amount of prenatal care. In this group 42% received methadone treatment. The daily contact with their treatment center facilitated their compliance with prenatal care.

Cigarette smoking. There was a high incidence of cigarette smoking reported among all patients. The difference between toxicology-positive patients and controls, however, was significant. Multichemical users had the highest incidence of smoking (Table III).

Alcohol use. The true level of alcohol use in our patient population was not ascertained because testing was not performed for alcohol and detailed information concerning quantity and frequency was not recorded in a significant number of charts. However, 37.3% of toxicology-positive patients reported alcohol use, compared with 10.6% of controls. This represented a statistically significantly different level of self-reported use. Cocaine and multichemical users were more likely than amphetamine users to report alcohol use (Table III).

Drug history. There was substantial denial of chemical use among the toxicology-positive patients. Denial of *any* chemical use at *any* time was obtained from 38.5% of these women (Table III). It is important to note that 25% of the women with a negative toxicology test reported chemical use before or during the index pregnancy.

Pregnancy complications. The incidence of pre-

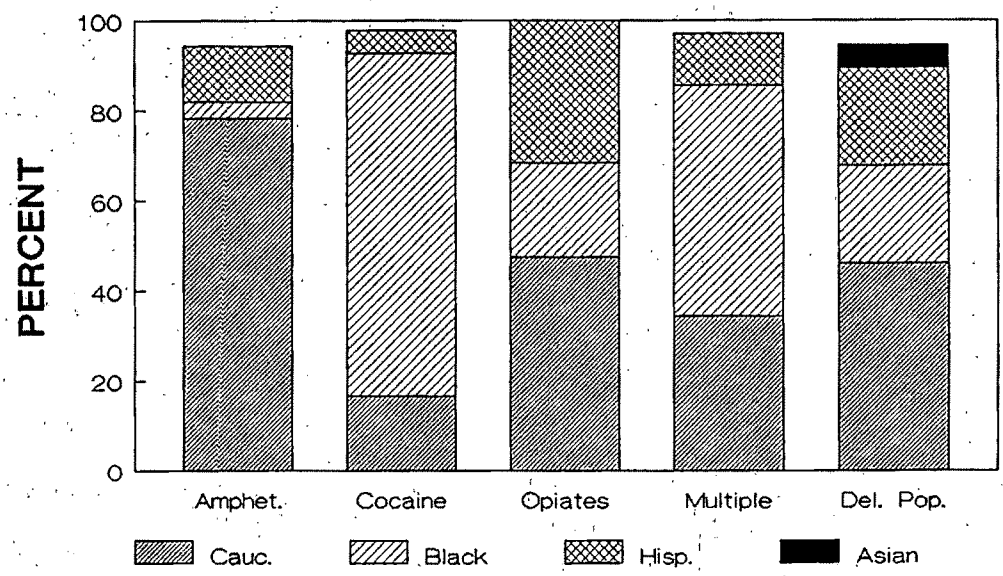


Fig. 1. Comparison of ethnic backgrounds of amphetamine, cocaine, opiate, or multiple substance users as detected by urine toxicology screen on admission to the obstetric service and the overall obstetric population.

Table II. Prenatal care by group and category

	Controls (n = 293) (%)	*Cases (n = 299) (%)*	*Cocaine (n = 139) (%)*	*Amphetamines (n = 106) (%)*	*Multichemicals (n = 35) (%)*	*Opiates (n = 19) (%)*
1st trimester	13	7*	6	6	11	21
2nd trimester	35	24	25	18	26	47
3rd trimester	30	25	21	27	20	21
None	11	38†	40†	40†	40†	11
Unknown onset	11	6	9	3	3	0

*$p < 0.05$, comparison with controls.
†$p < 0.01$, comparison with controls.

Table III. Smoking, alcohol, and drug history

	Controls (%)	*Cases (%)*	*Cocaine (%)*	*Amphetamines (%)*	*Opiates (%)*	*Multichemicals (%)*
Smokers ($n = 592$)	32	78*	74*	80*	58	94*
Alcohol						
Pregnancy ($n = 584$)	11	37*	48*	22†	32	46*
Drug history						
Pregnancy	‡	52	56	42	68	57
Past only ($n = 297$)	‡	10	12	6	10	14

*$p < 0.001$, comparison with controls.
†$p < 0.05$, comparison with controls.
‡All patients with drug history excluded during control selection process.

mature labor was significantly increased in the chemical-positive group as compared with the chemical-negative group ($p < 0.006$). The incidence of premature labor was highest in cocaine and multiple chemical users.

The incidence of premature rupture of membranes, abruptio placentae, intrauterine fetal death, and fetal distress was not significantly different between the two groups. Although cocaine and multiple chemical users had more frequent occurrences of these complications, the differences were not statistically significant on comparison with control patients (Table IV). Congenital

Table IV. Pregnancy complications

Complication	Controls (n = 293) (%)	Cases (n = 299) (%)	Amphetamines (n = 106) (%)	Cocaine (n = 139) (%)	Opiates (n = 19) (%)	Multichemicals (n = 35) (%)
Preterm labor	12.6	21.1*	16.0	25.2*	10.5	25.7
Premature rupture of membranes	7.5	9.7	4.7	12.2	0	20.0
Abruptio placental	0.7	1.3	0.9	0.7	0	5.7
Intrauterine fetal death	1.4	2.0	0	2.2	0	8.6
Fetal distress	5.5	7.0	3.7	8.6	5.3	11.4
Congenital anomalies	2.7	2.0	0.9	3.0	0	2.9

*$p < 0.001$, comparison with controls.

Table V. Method of delivery

Method	Controls (n = 299) (%)	Cases (n = 293) (%)	Amphetamines (n = 106) (%)	Cocaine (n = 139) (%)	Opiates (n = 19) (%)	Multichemicals (n = 35) (%)
Spontaneous vaginal	83	83	84	86	74	77
Primary cesarean section	11	7	7	8	16	3
Repeat cesarean section	3	4	5	4	5	3
Forceps	2	3	2	0	5	6
Vacuum	1	1	1	1	0	0
Vaginal breech	1	2	2	1	0	11*

*$p < 0.05$, comparison with controls (Bonferroni correction applied, Fisher's exact test).

anomalies were not increased in the toxicology-positive group. One case of hypospadias was noted in each of the amphetamine group and the control group.

Method of delivery. There was no significant difference in delivery method between toxicology-negative and toxicology-positive groups (Table V). There was a difference when chemical categories were analyzed separately ($p < 0.01$), with an increase in breech vaginal delivery among multiple chemical users.

Birth outcomes. Birth weight, length, head circumference, and gestational age were significantly lower in the toxicology-positive group as compared with the negative group. This difference was most marked in the cocaine and multiple chemical users. The group that was positive for opiates alone had outcomes that were similar to those of controls (Table VI).

The difference persisted for birth weight, length, head circumference, and gestational age when differences in prenatal care, smoking, and prior preterm births were controlled (Table VII). With control for gestational age, significant differences remained between groups for birth weight and head circumference. With analysis of covariance, there was a significant interaction between group and gestational age (Fig. 2).

Low birth weight (<2500 gm) and prematurity (<37 weeks) were significantly increased in the toxicology-positive group ($p < 0.002$). Low birth weight at term (≥37 weeks) was also increased in the toxicology-positive group ($p < 0.01$). These differences persisted in the cocaine and multiple chemical groups when these groups were analyzed separately. Case Apgar scores tended to be lower than control scores at 1 and 5 minutes ($p = 0.08$ and $p = 0.025$, respectively).

Comment

Most previous studies of perinatal chemical use have reported data from risk- or complication-directed toxicology testing. Selective screening by any criteria has the inherent risk of underdetection of the toxicology-positive group and consequent misrepresentation of the perinatal results and complications related to chemical use. Additionally, the influence of confounding factors is impossible to assess with selective testing since the population is not defined and the distribution of all potential factors of influence is not known.

This study examines the prevalence of cocaine, amphetamine, and opiate use, detected by universal urine testing in a defined obstetric population. The criteria for testing were simply admission to the Obstetrics Service at UCDMC. The majority of women were in labor.

Table VI. Perinatal outcome

	Controls	*Cases*	*Amphetamines*	*Cocaine*	*Opiates*	*Multichemicals*
Birth weight (mean, gm) (n = 583)	3165.6	2785.8*	294.7†	2682.6*	3026.7	2575.0*
Length (mean, cm) (n = 565)	48.8	47.0*	48.0	46.3*	49.3	45.9*
Head circumference (mean, cm) (n = 569)	33.5	32.3*	32.8†	31.9*	33.4	32.2†
Gestational age (obstetric) (mean, wk) (n = 591)	38.4	37.2*	37.8	36.9*	38.7	36.1*
Gestational age (pediatric) (mean, wk) (n = 564)	38.7	38.0†	38.1	37.8†	39.1	37.5
Premature birth (<37 wk) (%)	16	27*	19	32*	16	40*
Low birth weight (<2500 gm) (%)	14	26*	17	32*	16	36*

*p < 0.01, comparison with controls.
†p < 0.05, comparison with controls.

Table VII. Multiple regression analysis—Regression coefficients and p values

	Birth weight		*Head circumference*		*Length*	
Variable	*Regression coefficient*	*p*	*Regression coefficient*	*p*	*Regression coefficient*	*p*
Constant	3241		33.8		49.2	
Toxicology positive (0,1)	−300	<0.001	−1.0	<0.001	−1.3	0.008
Smoking (0,1)	−129	0.061	−0.4	0.078	−0.9	0.064
Prior preterm birth	−250	<0.001	−0.8	<0.001	−1.5	<0.001
Trimesters of prenatal care	−11	0.745	0.0	0.830	0.2	0.464

The peripartum chemical use rate was 20.5%. Nineteen percent of patients admitted to the Obstetrics Service did not have a toxicology result available for reasons such as failure to provide a urine sample before delivery, lack of patient cooperation, refusal to provide urine, and lost specimens. Even in the unlikely event that all of these patients had had negative results, the peripartum prevalence rate would have been 16.6%. We suspect that the positive rate of the untested patients was at least equal to the group for which test results were available.

This study confirms previous suggestions that a drug history alone is not accurate for identifying chemical use among pregnant women. Almost half of the women with positive tests denied chemical use during pregnancy.

One limitation of this study is that chemical use was ascertained from a test on one occasion, linked to hospital admission. Some "controls" might have used cocaine, amphetamines, or opiates earlier in pregnancy. Testing for marijuana was not performed because of limited laboratory capabilities and cost considerations. If testing for marijuana, nicotine, and alcohol were added, it is virtually certain the number of positive tests would have increased significantly. While we believe the use of other potent chemicals such as LSD, PCP, and prescription drugs is relatively low in our population, these drugs are certainly not absent.

Although cocaine use is spreading rapidly throughout the United States, amphetamine use is recognized to be more characteristic of the West Coast and of northern California in particular. Local manufacture of amphetamines is common in northern California. The drug is often used as an alternative to cocaine and generally appears to supplant cocaine use in our population. With the recent introduction of crystal methamphetamine ("ice"), it is anticipated that more widespread use of amphetamines will occur throughout the United States.

This study demonstrated significant differences in several demographic factors between the toxicology-positive and toxicology-negative groups of pregnant women. Chemical-positive women were older, were of higher gravidity, and had much less prenatal care than

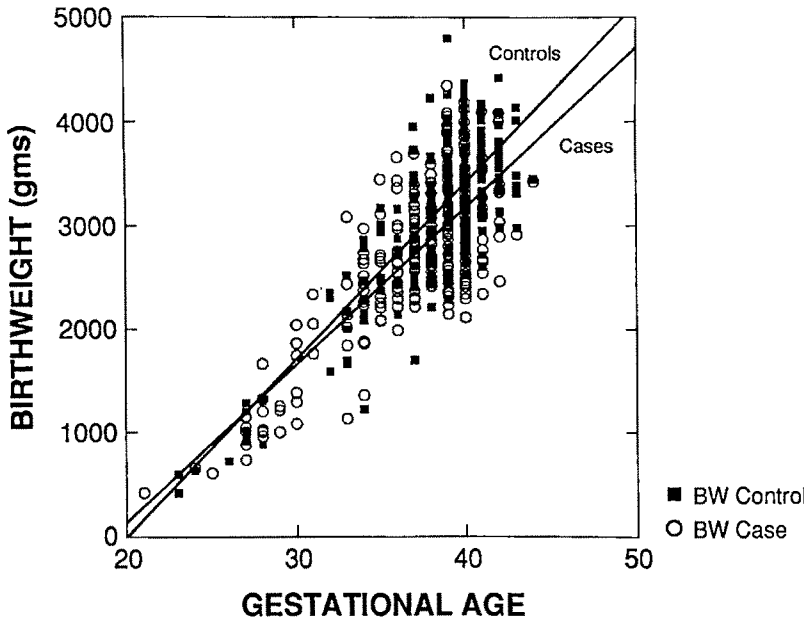


Fig. 2. Relationship of birth weight and gestational age in control patients (toxicology screen and drug history negative) and cases (toxicology screen positive).

the control patients. It appears from these data that these patients are not simply young, vulnerable, and inexperienced women who are casually experimenting with these chemicals. The greater mean age and parity, compared with those of the control group, suggest the possibility of a more chronic or long-standing pattern of chemical use in these women. Progression to cocaine and amphetamine usually occurs after a period of alcohol, cigarette, and marijuana use.[7] The pattern of progressive chemical use suggests that many of these women who are identified as cocaine or amphetamine users today may progress to the use of narcotics to counteract the intense highs of cocaine and amphetamines.

The differences in smoking and alcohol use between the two groups were remarkable. Smoking proved to be the single finding most strongly correlated with a positive urine toxicology result. It is highly likely that the actual use of alcohol among all groups was substantially greater than reported by patients. Many women recognize that alcohol should be avoided during pregnancy. The degree of denial for cocaine, amphetamine, and opiate use among toxicology-positive patients suggests that the same situation exists with the reporting of alcohol use.

There were significant variations in race among the toxicology-positive patients when analyzed by the drug for which they were positive. These racial differences in the "drug of choice" are likely to be regional and may vary widely.

The finding that toxicology-positive patients were less likely to be married has important implications. A large number of chemically positive mothers are likely to be parenting alone or with inconsistent support from partners. We were not able to evaluate the level of family support available to these women, but these findings suggest a need for greater social and economic support services.

Although factors that place the pregnancy at risk such as smoking, limited prenatal care, and prior preterm births were increased in the toxicology-positive group, there remained a significant direct adverse effect of substance abuse on birth weight, head circumference, length, gestational age, and Apgar scores. For length and Apgar scores, this effect appeared to be mediated through the decreased gestational age. However, for birth weight and head circumference, there was a decrease that was independent of gestational age. This difference in birth weight increased with gestational age. It appears that head circumference is not spared with the growth retardation associated with chemical abuse.

The incidence of premature rupture of the membranes did not statistically increase in the toxicology-positive group. This supports the theory that the mechanism for premature labor associated with chemical use is related to increased uterine irritability in association with vascular spasm or a direct effect on myometrium instead of an infectious cause. Abruptio placentae was not increased significantly, in contrast to some previous reports.[4, 6] Reports that have demonstrated such an increase have used selective screening, possibly under-

representing the incidence of substance abuse in the general obstetric population. Alternatively, the failure to detect an increase in abruptio placentae in our study and another population study[8] may be a type II error resulting from the low frequency of the complication in either group.

The incidence of congenital anomalies was similar in the two groups. Previous studies have reported conflicting results. Zuckerman et al.[1] found a marked increase in major or multiple anomalies in cocaine users, without an increase in overall incidence of anomalies. Neerhof et al.[8] found no increase in anomalies in infants exposed to cocaine only but did find an increase with exposure to multiple substances. Others[9, 10] have found an increase in genitourinary anomalies with first-trimester cocaine use. No increases in such anomalies were noted in this study. Neonatal renal ultrasonography was not performed routinely; however, antenatal ultrasonography was performed in the majority of patients receiving prenatal care.

The findings in this study have several important therapeutic implications. Because chemical use has an independent adverse effect on pregnancy outcome, identification and intervention early in the pregnancy offer the mother and fetus the greatest potential for a good pregnancy outcome.[6, 11] Women engaging in perinatal chemical use often have additional risk factors such as smoking, alcohol use, and prior preterm birth. These need to be addressed in a comprehensive fashion during obstetric care. Patient education should include information about the warning signs of preterm labor and instruction for self-palpation of uterine contractions.[12] Serial ultrasonography should be used to evaluate fetal anatomy and growth. Antenatal fetal testing is used for the usual obstetric indications.

Although not addressed in this study, decreased pregnancy weight gain has been reported in cocaine and marijuana users.[1] Birth weight and gestational age were increased in a group of women with poor weight gain who were subsequently enrolled in an intensive nutrition intervention program, on comparison with a similar group of controls receiving routine prenatal care.[13] Thus intense nutritional surveillance and education should be a part of the obstetric management of the pregnant substance abuser.

In summary, with universal urine toxicology testing on admission to an obstetrics service, we documented a significant level of cocaine, amphetamine, and opiate use. Smoking, limited prenatal care, alcohol use, and prior preterm births were significantly increased in this group of women, placing the pregnancy at risk for complications in addition to the direct effects of chemical use. An independent, adverse effect of these chemicals on fetal size and gestational age at delivery was found. The effect on fetal size appears to be mediated through both preterm birth and growth retardation. Comprehensive outreach, identification, and treatment programs are required to prevent the consequences of perinatal chemical use.

We thank Sunny Eidson, Elizabeth Perry, Carolyn Hughes, and Gary Hughes for their assistance.

REFERENCES

1. Zuckerman B, Frank DA, Hingson R, et al. Effects of maternal marijuana and cocaine use on fetal growth. N Engl J Med 1989;320:762-8.
2. Chasnoff IJ, Bussey ME, Savich R, Stack CM. Perinatal cerebral infarction and maternal cocaine use. J Pediatr 1986;108:456-9.
3. Chasnoff IJ, Hunt CE, Kletter R, Kaplan D. Prenatal cocaine exposure is associated with respiratory pattern abnormalities. Am J Dis Child 1989;143:583-7.
4. Oro AS, Dixon SD. Perinatal cocaine and methamphetamine exposure: maternal and neonatal correlates. J Pediatr 1987;111:571-8.
5. Pelosi MA, Frattarola M, Apuzzio J, et al. Pregnancy complicated by heroin addiction. Obstet Gynecol 1975; 45:512-5.
6. Chasnoff IJ, Griffith DR, MacGregor S, Dirkes K, Burns K. Temporal patterns of cocaine use in pregnancy. JAMA 1989;261:1741-4.
7. Yamaguchi K, Kandel DB. Patterns of drug use from adolescence to young adulthood. II. Sequences of progression. Am J Public Health 1984;74:668-72.
8. Neerhof MG, MacGregor SN, Retzky SS, Sullivan TP. Cocaine abuse during pregnancy: peripartum prevalence and perinatal outcome. Am J Obstet Gynecol 1989; 161:633-8.
9. Chasnoff IJ, Chisum GM, Kaplan WE. Maternal cocaine use and genitourinary tract malformations. Teratology 1988;37:201-4.
10. Chavez GF, Mulinarre J, Cordero JF. Maternal cocaine use during early pregnancy as a risk factor for congenital urogenital anomalies. JAMA 1989;262:795-8.
11. Connaughton JF, Reeser D, Schut J, Finnegan LP. Perinatal addiction: outcome and management. Am J Obstet Gynecol 1977;129:679-86.
12. Mueller-Heubach E, Reddick D, Barnett B, Bente R. Preterm birth prevention: evaluation of a prospective controlled randomized trial. Am J Obstet Gynecol 1989; 160:1172-8.
13. Bruce L, Tchabo J. Nutrition intervention program in a prenatal clinic. Obstet Gynecol 1989;74:310-2.

Prenatal diagnosis of fetal intracranial calcifications

Yasutsugu Koga, MD, Masahiko Mizumoto, MD, Yoshinori Matsumoto, MD, Tetsuo Hattori, MD, Shinichi Tanaka, MD, Toshinobu Tanaka, MD, and Seiichiro Fujimoto, MD

Sapporo, Japan

A case is presented wherein fetal intracranial calcifications as a result of intrauterine cytomegalovirus infection were visualized by computed tomography and magnetic resonance imaging at 36 weeks' gestation. Diagnosis of in utero cytomegalovirus infection was made by isolation of the virus from amniotic fluid. No symptomatic abnormality has been noted in the neonatal period except periventricular calcifications. (Am J Obstet Gynecol 1990;163:1543-5.)

Key words: Intracranial calcification, congenital cytomegalovirus infection, fetal cranial computed tomography and magnetic resonance imaging, prenatal diagnosis

Fetal intracranial calcifications are rare findings and are difficult to clarify by antenatal ultrasonography. We present a case in which periventricular calcifications as a result of intrauterine cytomegalovirus infection were clearly visible by fetal cranial computed tomography and magnetic resonance imaging.

Case report

A 29-year-old Japanese woman, gravida 2, para 2, was referred to our clinic at 36½ weeks' gestation because a previous ultrasonographic examination performed elsewhere had identified ventriculomegaly. The course of pregnancy had been uneventful and, in particular, there was no history of fever, rash, and malaise.

Our ultrasonogram revealed a single fetus with biometry that was consistent with gestational age. Intracranial scans showed bilateral dilatation of the lateral ventricles. Although small areas close to the periventricular wall appeared hyperechogenic, we could not discriminate between calcifications and artifacts. To confirm whether calcifications existed, we performed cranial computed tomography and magnetic resonance imaging of the fetus.

Computed tomography visualized discrete high-density spots scattered along the periventricular wall (Fig. 1). On magnetic resonance imaging, a high-intensity area formed on part of the wall of the left lateral ventricle with dilatation was clearly visible by means of T_1-weighted images (600 msec repetition-time, 15 msec echo-time) (Fig. 2). Both findings were diagnosed as foci of calcifications. An intrauterine cytomegalovirus infection was thought to be the most likely cause of these images.

On maternal serologic screening for infections, the hemagglutination inhibition test for rubella was positive at 1:8, and both toxoplasma immunoglobulin (Ig) G and IgM antibodies were negative by fluorescent antibody technique. The cytomegalovirus titer with enzyme-linked immunosorbent assay (ELISA) was IgM-negative and IgG-strongly positive. Transabdominal amniocentesis performed for a viral culture yielded a positive culture for cytomegalovirus.

At 39½ weeks' gestation a 3020 gm male infant with an Apgar score of 8 at 1 minute was delivered by induction of labor. Bilateral dilatation of the lateral ventricles and periventricular calcifications were verified in the neonate by computed tomography, but no other abnormalities such as hepatosplenomegaly, hyperbilirubinemia, petechial rash, microcephaly, or chorioretinitis were noted. Both IgG and IgM antibodies for cytomegalovirus with enzyme-linked immunosorbent assay were positive in the cord blood, and intrauterine cytomegalovirus infection was identified on the basis of viruria detected at birth. At 12 months of age the infant showed signs of psychomotor retardation with a spastic tetraplegia.

Comment

Cytomegalovirus is known to be one of the main causes of congenital infections. Although 95% of pregnant Japanese women are immune to cytomegalovirus, the incidence of intrauterine cytomegalovirus infection in Japan is about 0.5%, similar to that reported in Europe and America.[1]

Clinical features of congenital cytomegalovirus infection vary, ranging from a silent infection to classic cytomegalic inclusion disease. Periventricular calcifi-

From the Division of Obstetrics, Hokkaido University School of Medicine.
Received for publication April 25, 1990; accepted May 24, 1990.
Reprint requests: Yasutsugu Koga, MD, Division of Obstetrics, Hokkaido University School of Medicine, Sapporo, Japan 060.
6/1/22722

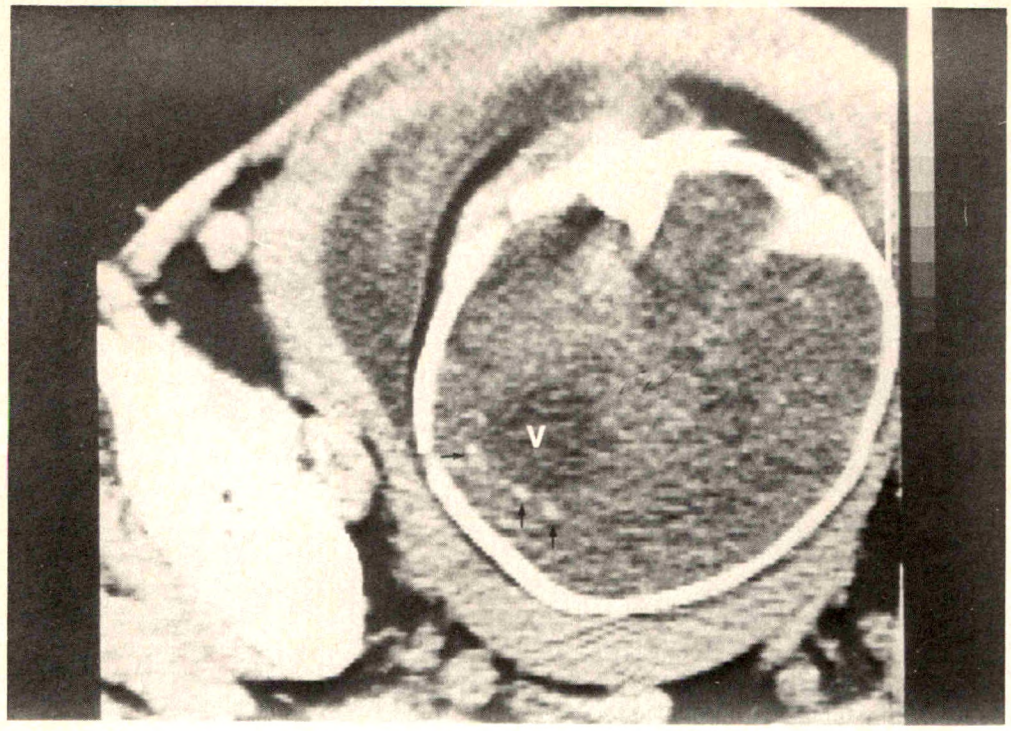


Fig. 1. Fetal cranial computed tomography. Enlargement of left lateral ventricle *(V)* and discrete high-density spots *(arrows)* scattered along periventricular wall are clearly visible.

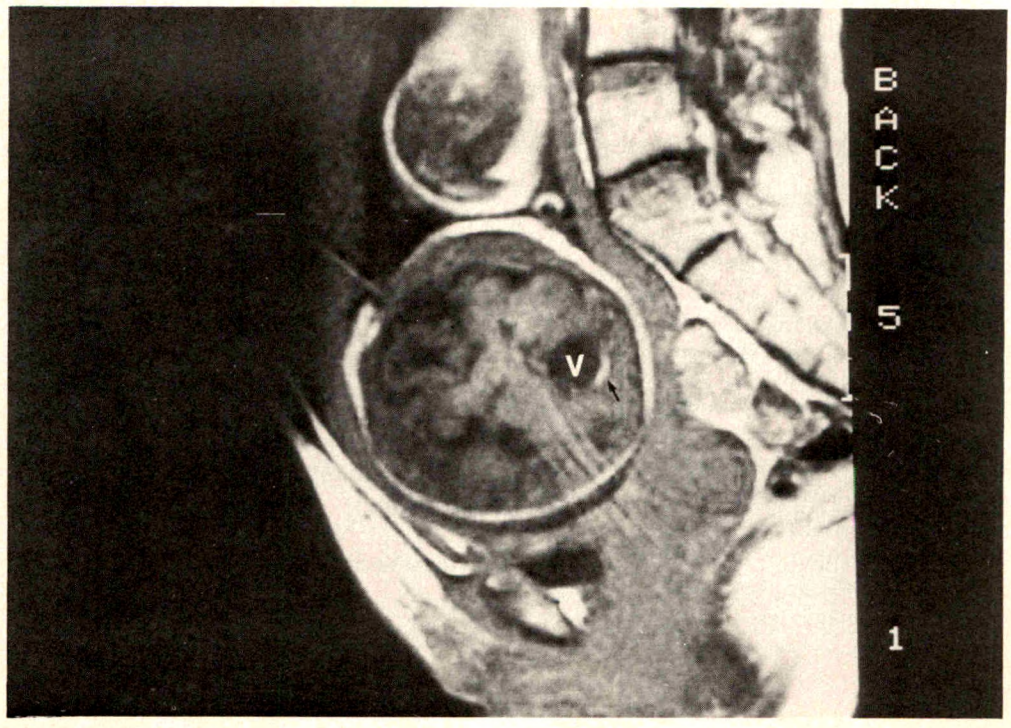


Fig. 2. T_1-weighted magnetic resonance imaging (600 msec repetition-time, 15 msec echo-time) of fetal brain. High-intensity area *(arrow)* forms part of wall of left lateral ventricle with dilatation *(V)*.

cations, characteristic of severe congenital cytomegalovirus encephalitis, are frequently observed with microcephaly. Besides cerebral calcifications, in our case the infant manifested not only microcephaly but other clinical features of symptomatic congenital cytomegalovirus infection in the neonatal period. Therefore the neonate probably would be recognized as a healthy newborn infant if intrauterine cytomegalovirus infection was not prenatally proved.

It is possible that prenatal diagnosis of congenital cytomegalovirus infection can be made by recovery of the virus from amniotic fluid.[2] At present there are a few reports in the literature that describe the isolation of cytomegalovirus from amniotic fluid. However, we are not aware of a case previously reported in which fluid from an amniocentesis performed on a pregnant woman without a symptomatic cytomegalovirus infection because of fetal periventricular calcifications was positive for cytomegalovirus, as was found in this case.

Fetal cerebral calcifications are very difficult to identify with ultrasonography because flecks of calcifications are too small to cause acoustic shadowing. In our case, fetal cranial computed tomography visualized foci of periventricular calcifications. On magnetic resonance imaging, T_1-weighted images clearly showed an area of high intensity along the periventricular wall, although magnetic resonance imaging is generally unfavorable to show calcifications because of the low proton density of calcium.

On the basis of our experience, the combination of fetal intracranial ultrasonography and magnetic resonance imaging is of clinical value to establish a less invasive diagnosis of cerebral calcifications.

REFERENCES

1. Chiba S, Kamada M, Yoshimura H, Hiraki M, Nakao T. Congenital cytomegalovirus infection in Japan. N Engl J Med 1984;310:50.
2. Congenital cytomegalovirus. In: Hanshaw JB, Dudgeon JA, Marshall WC, eds. Viral diseases of the fetus and newborn. Philadelphia: WB Saunders, 1985:92-131.

Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast

Marie R. Rodway, MSc, Basil Ho Yuen, MB, and Peter C. K. Leung, PhD
Vancouver, British Columbia, Canada

We have previously shown that 8-bromo-adenosine-3′:5′-cyclic monophosphate inhibits production of estradiol from androgen precursors in trophoblast isolated from term placentas. In these experiments we sought to evaluate the effect of 8-bromo-adenosine-3′:5′-cyclic monophosphate on aromatase activity in first-trimester trophoblast culture. Trophoblast cells were enzyme dispersed from first-trimester placentas and purified on a Percoll gradient. On the third and fourth day after dispersion cultures were treated with androstenedione or testosterone with and without 2 mmol/L 8-bromo-adenosine-3′:5′-cyclic monophosphate. The quantity of estradiol, estrone, progesterone, human placental lactogen, and human chorionic gonadotropin was determined by radioimmunoassay. We concluded that in first-trimester trophoblast, as previously reported in term trophoblast, 8-bromo-adenosine-3′:5′-cyclic monophosphate inhibited the production of estradiol from the supplied androgen precursors. Human chorionic gonadotropin and human placental lactogen production was stimulated by 8-bromo-adenosine-3′:5′-cyclic monophosphate. Progesterone production was either stimulated or was unchanged by the presence of 8-bromo-adenosine-3′:5′-cyclic monophosphate. (AM J OBSTET GYNECOL 1990;163:1546-51.)

Key words: Placental cell culture, aromatase, cyclic adenosine monophosphate, trophoblast

During pregnancy the placenta is the primary site of estrogen synthesis after the first trimester. The placenta is deficient in $C_{17,20}$-lyase and 17α-hydroxylase, and is therefore dependent on androgen synthesis, primarily in the fetal compartment, for precursors that are aromatized to estrogens in the placenta.

Cyclic adenosine monophosphate is a well-known second messenger that affects hormonogenesis in cultured trophoblast. Stimulation[1-3] or inhibition[4,5] of aromatase activity has been reported in response to cyclic adenosine monophosphate treatment of trophoblast in culture.

Cyclic adenosine monophosphate also affects the production of human chorionic gonadotropin (hCG), progesterone, and human placental lactogen (hPL). The production of hCG in cultured cells is stimulated by treatment with cyclic adenosine monophosphate,[4-8] as is the production of progesterone.[5,6,8] Production of hPL by cultured cells has been reported to be stimulated,[9,10] inhibited,[2,11] or unaffected[12] by the presence of cyclic adenosine monophosphate.

Hormone production and regulation of this production in the trophoblast is believed to be dependent on the gestational age of the placenta.[13] Some studies have sought to determine the effect of cyclic adenosine monophosphate in cell cultures prepared from first-trimester placentas. Human chorionic gonadotropin production was stimulated by cyclic adenosine monophosphate in cultured cells from first-trimester placentas.[14]

In this study we examined the effect of 8-bromo-adenosine-3′:5′-cyclic monophosphate (8-br-cAMP) on the production of estradiol and estrone from androgen (androstenedione, testosterone) precursors in first-trimester human trophoblast cell cultures. For comparison, the effects of 8-br-cAMP on hCG, hPL, and progesterone output by the cells were also determined.

Methods and material

Trophoblast cells were prepared according to the method of Kliman et al.[15] with some modifications. Tissue was collected immediately after vacuum aspiration. Membranes were excluded and trophoblast tissue was finely minced and digested in Hanks' balanced salt solution (Gibco Laboratories, St. Lawrence, Mass.) containing 0.25% trypsin (Gibco) and 500 U/dl of deoxyribonuclease type I (Sigma Chemical Company, St. Louis) at 37° C for five 10-minute digestions. After each digestion the supernatant was centrifuged at 1000

From the Department of Obstetrics and Gynecology, University of British Columbia, Grace Hospital.
Supported by the British Columbia Health Care Research Foundation. P.C.K.L. was the recipient of a Medical Research Council Scientist Award.
Received for publication February 21, 1990; revised July 12, 1990; accepted July 20, 1990.
Reprint requests: P.C.K. Leung, PhD, Department of Obstetrics and Gynecology, University of British Columbia, Grace Hospital, Vancouver, British Columbia, Canada V6H 3V5.
6/1/24039

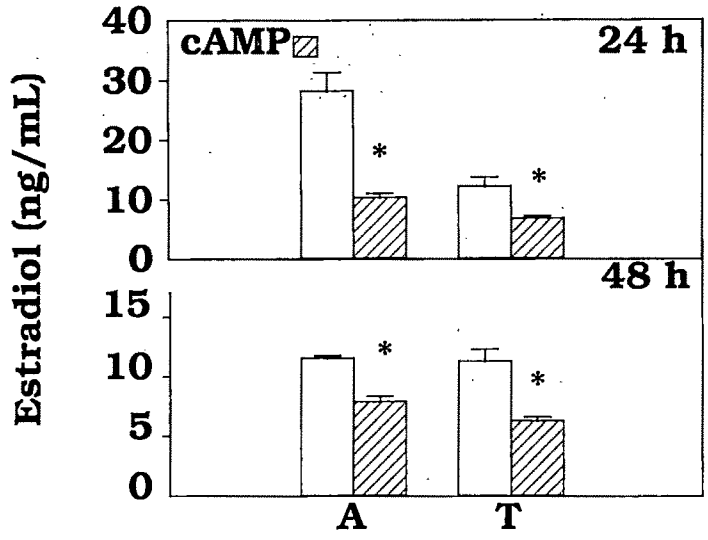


Fig. 1. Effect of 8-br-cAMP on production of estradiol from androstenedione or testosterone by cultured first-trimester trophoblast in culture in presence of 10% fetal calf serum. This figure is representative of four experiments that produced similar results. *Asterisk*, $p < 0.01$, androgen versus androgen with 8-br-cAMP.

g for 10 minutes over 1 ml of calf serum. Collected cells were pooled and placed on a Percoll gradient. Cytotrophoblast were collected and centrifuged at 1000 *g* to remove Percoll and were resuspended in Dulbecco's modified Eagle medium (Gibco) supplemented with 10% fetal calf serum, and then counted and plated in 24 multiwell culture plates (Falcon, Becton-Dickinson, Rutherford, N.J.) at a density of 0.5 million cells/ml/well. Cells were cultured for 24 hours (37° C, 5% carbon dioxide in air) and the media was then changed to Dulbecco's modified Eagle medium without serum supplement (designated as "serum-free" cultures), or to Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. Cultures were treated at 48 and 72 hours and media were collected at 72 and 96 hours. The medium was stored at −20° C until hormones could be analyzed by radioimmunoassays. Experiments were done in quadruplicate and four experiments with and without serum supplement were done. hCG was analyzed by means of β-hCG kits (Amerlex, purchased from Amersham, Arlington Heights, Ill.). Estradiol, estrone, and progesterone concentrations were determined by radioimmunoassay with antisera provided by D. T. Armstrong (University of Western Ontario). hPL was analyzed by radioimmunoassay with hPL and hPL antiserum supplied by the National Institute of Diabetes Research. The percentage of intraassay and interassay variance, respectively, was 8.4% and 12.2% for estradiol, 7.0% and 7.4% for estrone, 6.4% and 7.9% for progesterone, 5.2% and 7.3% for hCG, and 6.6% and 10.4% for hPL.

Results

In four experiments in which 10% fetal calf serum was present during the treatment period, there was an apparent suppression of aromatase activity in the presence of 2 mmol/L 8-br-cAMP. In all cases there was a significant ($p < 0.01$) decrease in the conversion of androgen precursors (androstenedione and testosterone) to estradiol (Fig. 1). Cultures were treated 48 and 72 hours after plating and the suppression was evident after the first 24 hours of exposure to 8-br-cAMP. In three of these four experiments a significant suppression of estrone formation also occurred (Fig. 2). In serum-free cultures, this effect on the production of estradiol was observed in three of the four experiments after 24 hours' exposure to 8-br-cAMP, but not after 48 hours (data not shown). Decreased production of estrone from androstenedione and testosterone was seen after 48 hours of culture in only one experiment (data not shown).

Addition of 2 mmol/L 8-br-cAMP stimulated production of hPL. In the presence of fetal calf serum, this increase occurred in one of three cultures at 24 hours and in all at 48 hours (Fig. 3). In the serum-free cultures, hPL was significantly increased at 24 hours in three of four cultures and in all cultures by 48 hours of exposure to 8-br-cAMP (data not shown). There was no apparent effect of the presence of androstenedione or testosterone on the production of hPL.

8-Br-cAMP significantly stimulated production of hCG in the presence and absence of serum in all but one experiment at 24 and 48 hours of treatment (Fig.

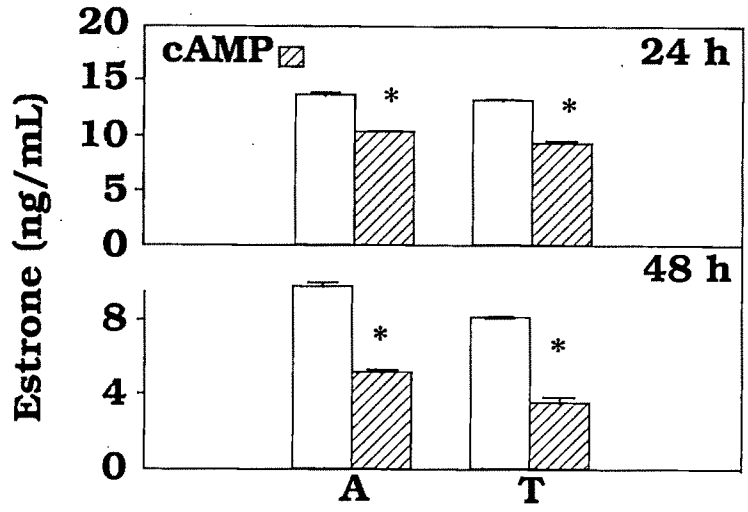


Fig. 2. Effect of 8-br-cAMP on the production of estrone from androstenedione or testosterone by cultured first-trimester trophoblast in culture in presence of 10% fetal calf serum. This figure is representative of four experiments. In three of four experiments significant suppression of estrone formation was seen. *Asterisk*, $p < 0.01$, androgen versus androgen with 8-br-cAMP.

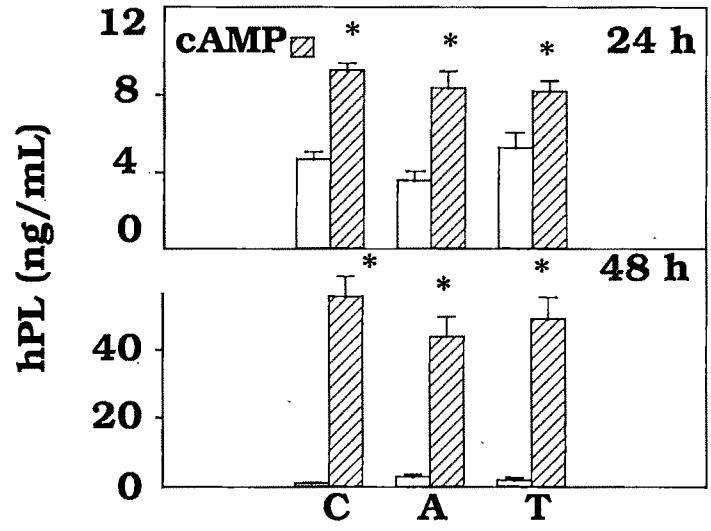


Fig. 3. Effect of 8-br-cAMP on production of hPL by cultured first-trimester trophoblast in the presence of 10% fetal calf serum. This figure is representative of three experiments. In the remaining experiments a significant stimulation of hPL production was not seen until cells had been exposed to 8-br-cAMP for 48 hours. *Asterisk*, $p < 0.01$, control versus 8-br-cAMP treated.

4). Significant stimulation of progesterone production occurred in only two of four cultures in which 10% serum was used (stimulation of progesterone did not occur in this culture, Fig. 5) and in three of four serum-free cultures (at 24 and 48 hours of treatment; data not shown). There was no apparent effect of the presence of androstenedione or testosterone on the production of hCG or progesterone.

Comment

As previously reported in first-trimester trophoblast cell cultures,[14] cyclic adenosine monophosphate caused increased production of hCG in this study. Similarly, the stimulatory effect of 8-br-cAMP on hPL production has been reported in term placental cell culture[9, 10] and has proved consistent in our study of first-trimester cultures prepared in the same manner.[8] The stimulatory effect of cyclic adenosine monophosphate on progesterone production was not as consistent in our first-trimester trophoblast cultures, as has been reported in term trophoblast cultures.[5, 6, 8]

The effect of cyclic adenosine monophosphate on aromatase in first-trimester placental cultures and on term placental cell cultures as reported previously[4, 7, 8]

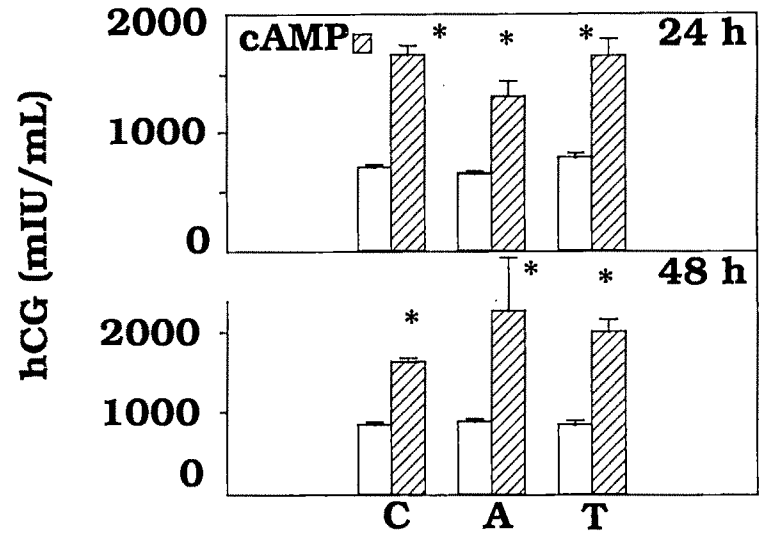


Fig. 4. Effect of 8-br-cAMP on production of hCG by cultured first-trimester trophoblast in presence of 10% fetal calf serum. This figure is representative of four experiments, three of which showed stimulation of hCG production in the presence of 8-br-cAMP. *Asterisk*, $p < 0.01$, control versus 8-br-cAMP treated.

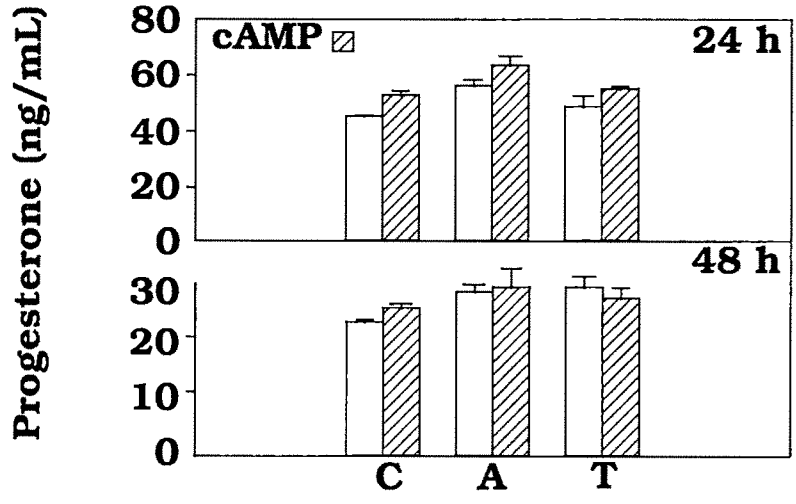


Fig. 5. Effect of 8-br-cAMP on production of progesterone by cultured first-trimester trophoblast in presence of 10% fetal calf serum. This figure is representative of four experiments. In two experiments stimulation of progesterone production occurred. *Asterisk*, $p < 0.01$, control versus 8-br-cAMP treated.

is in contrast to the effect seen in other cell types[16] and in term cells prepared in a manner almost identical to our own.[3] By use of a tritiated water release assay, Lobo and Bellino[3] found that cyclic adenosine monophosphate stimulated aromatase activity in cultured term placental cells. Corbin et al.[17] showed that only one gene codes cytochrome P_{450} aromatase; consequently, only one cytochrome P_{450} enzyme is produced. This eliminates the possibility of expression of different cytochrome P_{450} aromatase genes in different tissues as an explanation for these opposing results.

Fournet-Dulguerov et al.[18] used highly specific antibodies and found cytochrome P_{450} aromatase exclusively in syncytiotrophoblast. Although marked aromatase activity exists in freshly dispersed 95% pure cytotrophoblast,[15] unstimulated aromatase activity does increase by approximately fifteenfold from time of dispersion to 48 hours after dispersion.[3] It has been suggested that cyclic adenosine monophosphate accelerates "functional differentiation" from cytotrophoblast to syncytiotrophoblast.[6] Lobo and Bellino[3] treated cells 24 hours after dispersion and observed a stimulation

of aromatase activity, which could reflect increased functional differentiation. Our cells were initially exposed to cyclic adenosine monophosphate 48 hours after dispersion. Maximal morphologic syncytial formation occurs at 72 hours.[6] The cells may still have been subject to accelerated functional differentiation on exposure to cyclic adenosine monophosphate, but under such conditions stimulation and not the inhibition that was evident would be expected. It is possible that cyclic adenosine monophosphate has different effects that are dependent on the differentiation of the trophoblast.

Ringler et al.[19] studied the effect of 8-br-cAMP on production of the α subunit of hCG, hCG, and cytochrome P_{450} side chain cleavage enzyme messenger ribonucleic acids. They suggested that the mechanism(s) by which cyclic adenosine monophosphate affects hormonogenesis in trophoblast cells is via activation of a protein kinase that phosphorylates a protein that may act at cyclic adenosine monophosphate response elements in the involved genes. Evans et al.[20] have shown that in stromal adipose cells the level of messenger ribonucleic acid translation correlates with the activity of the aromatase system. Perhaps measurement of cytochrome P_{450} (aromatase) messenger ribonucleic acid in these cells will provide more information on the regulation of aromatase activity by cyclic adenosine monophosphate.

Mendelson et al.[16] previously investigated the effect of fetal calf serum, growth factors, and phorbol esters on aromatase activity in human adipose stromal cells. They found that cyclic adenosine monophosphate stimulated aromatase activity and that fetal calf serum attenuated the stimulatory effect of cyclic adenosine monophosphate on the aromatase activity. Epidermal, fibroblast, and platelet-derived growth factors also inhibited this stimulation. Phorbol esters potentiated the stimulatory effect of cyclic adenosine monophosphate on aromatase activity. Lobo and Bellino[3] found that the presence of serum was slightly additive to the stimulatory effect they observed. The presence of fetal calf serum in our cultures seemed to prolong the inhibitory effect of 8-br-cAMP on the inhibition of production of estradiol from androstenedione and testosterone.

In summary, our results indicate that in first-trimester trophoblast, cyclic adenosine monophosphate stimulates hCG and hPL production but suppresses the production of estradiol from androgens, presumably at the aromatization step. These effects are also seen in trophoblast prepared from term placentas, thus supporting a role of cyclic adenosine monophosphate as an intracellular regulator of placental hormonogenesis.

We thank Dr. David Chitayat for assistance in obtaining tissue samples, and Sandra Crespo for assistance in preparing the figures.

REFERENCES

1. Hochberg Z, Perlman R, Benderli A, Brandes JM. The effect of glucose, 2-deoxy-D-glucose and insulin on estradiol secretion by cultured human trophoblast. Biochem Biophys Res Commun 1982;108:102-9.
2. Hochberg Z, Perlman R, Bick T. Interrelated calcium ion and cyclic AMP inhibition of placental lactogen secretion by cultured human term trophoblast. Acta Endocrinol [Copenh] 1987;114:68-73.
3. Lobo JO, Bellino FL. Estrogen synthetase (aromatase) activity in primary culture of human term placental cells: effects of cell preparation, growth medium, and serum on adenosine 3′,5′-monophosphate response. J Clin Endocrinol Metab 1989;69:868-874.
4. Lobo JO, Bellino FL, Bankert L. Estrogen synthetase activity in human term placental cells in monolayer culture. Endocrinology 1985;116:889-95.
5. Zhou F, Ho Yuen B, Leung PCK. Effects of adenosine 3,5-cyclic monophosphate, forskolin, and cholera toxin on hormone production in human term placental cells. AM J OBSTET GYNECOL 1987;156:420-4.
6. Feinman MA, Kliman HJ, Caltabiano S, Strauss JF III. 8-Bromo-3′,5′-adenosine monophosphate stimulates the endocrine activity of human cytotrophoblast in culture. J Clin Endocrinol Metab 1986;63:1211-7.
7. Benoit J, Rodway M, Ho Yuen B, Leung PCK. Effects of cyclic adenosine monophosphate on human chorionic gonadotropin and estradiol output by cultured human placental cells. AM J OBSTET GYNECOL 1988;158:328-32.
8. Rodway MR, Zhou F, Benoit J, Ho Yuen B, Leung PCK. Differential effects of 8-bromo-cyclic AMP on human chorionic gonadotropin (hCG), progesterone and estrogen production by term placental cells. Life Sci 1988;43:1451-8.
9. Harman I, Costello A, Sane A, Handwerger S. Cyclic adenosine-3′,5′-monophosphate stimulates the acute release of placental lactogen from human trophoblast cells. Endocrinology 1987;121:59-63.
10. Wu SQ, Jorgensen EV, Handwerger S. High density lipoproteins stimulate placental lactogen release and adenosine 3′,5′-monophosphate (cAMP) production in human trophoblast cells: evidence for cAMP as a second messenger in human placental lactogen release. Endocrinology 1988;123:1879-84.
11. Hochberg Z, Bick T, Perlman R, Lahav M, Barzilai D. The modulation of placental lactogen secretion by calcium: studies with cultured human term trophoblast. Mol Cell Endocrinol 1984;37:359-62.
12. Zeitler P, Markoff E, Handwerger S. Characterization of the synthesis and release of human placental lactogen and human chorionic gonadotropin by an enriched population of dispersed placental cells. J Clin Endocrinol Metab 1983;57:812-8.
13. Siler-Khodr TM, Khodr GS, Rhode J, Vickery BH, Nestor JJ Jr. Gestational age-related inhibition of placental hCG, αhCG and steroid hormone release in vitro by a GnRH antagonist. Placenta 1987;8:1-14.
14. Yagel S, Lala PK, Powell WA, Casper RF. Interleukin-1 stimulates human chorionic gonadotropin secretion by first trimester human trophoblast. J Clin Endocrinol Metab 1989;68:992-5.
15. Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss JF III. Purification, characterization, and in vitro differentiation of cytotrophoblast from human term placentae. Endocrinology 1986;118:1567-82.
16. Mendelson CR, Cleland WH, Smith ME, Simpson ER. Regulation of aromatase activity of stromal cells derived from human adipose tissue. Endocrinology 1982;111:1077-85.
17. Corbin CJ, Graham-Lorence S, McPhaul M, Mason JI, Mendelson CR, Simpson ER. Isolation of a full-length cDNA insert encoding human aromatase system cyto-

chrome P-450 and its expression in nonsteroidogenic cells. Proc Natl Acad Sci USA 1988;85:8948-52.
18. Fournet-Dulguerov N, MacLusky NJ, Leranth CZ, et al. Immunohistochemical localization of aromatase cytochrome P-450 and estradiol dehydrogenase in the syncytiotrophoblast of the human placenta. J Clin Endocrinol Metab 1987;65:757-64.
19. Ringler GE, Kao L-C, Miller WL, Strauss JF III. Effects of 8-bromo-cAMP on expression of endocrine functions by cultured human trophoblast cells. Regulation of specific mRNAs. Mol Cell Endocrinol 1989;61:13-21.
20. Evans CT, Ledesma DB, Schulz TZ, Simpson ER, Mendelson CR. Isolation and characterization of a complementary DNA specific for human aromatase-system cytochrome P-450 mRNA. Proc Natl Acad Sci USA 1986; 83:6387-91.

A complete list of references on the subject of hormone production in the placenta is available from the authors on request.

A reappraisal of the need for autologous blood donation in the obstetric patient

Robert L. Andres, MD, Kathleen M. Piacquadio, MD, and Robert Resnik, MD
San Diego, California

There has been recent interest in autologous blood donation in obstetric patients, but little attention has been paid to whether such programs are needed or whether the patients that will require transfusion can be accurately predicted. At the University of California San Diego Medical Center from July 1 to Dec. 31, 1988, there were 2265 deliveries; 13 women (0.57%) received blood transfusions. Traditionally accepted risk factors were identified in 251 patients, with only four (1.6%) requiring transfusion. Among the 150 patients delivered by repeat cesarean section, only one (0.7%) required blood. One of 27 (3.7%) multiple gestations, two of eight (25%) patients with placenta previa, and none of the 66 grandmultiparous women had transfusions. These data suggest that autologous blood donation may not be beneficial or cost effective when the low frequency of blood transfusions in this high-risk obstetric population and the difficulty in accurately predicting those likely to require transfusions are considered. (Am J Obstet Gynecol 1990;163:1551-3.)

Key words: Transfusion, hemorrhage, obstetrics

Concern over the transmission of hepatitis and human immunodeficiency virus via the transfusion of homologous blood products is primarily responsible for the interest in the autologous donation of blood. The number of autologous donation programs nationwide increased fourfold from 1974 to 1982.[1] The predepositing of blood by obstetric patients was reported by Miles et al.[2] in 1986, and the focus of the literature since that time has been the issue of safety for the mother and fetus.[3-5] Although the safety question has been addressed, the need for such a program is uncertain. The purposes of our investigation were to determine the frequency of blood product use in a high-risk obstetric service and to evaluate the utility of standard risk factors in identification of patients who require transfusion.

From the Division of Perinatal Medicine, Department of Reproductive Medicine, University of California, San Diego.
Received for publication March 27, 1990; revised June 18, 1990; accepted July 27, 1990.
Reprint requests: Robert L. Andres, MD, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Health Science Center at Houston, 6431 Fannin, Suite 3.286, Houston, TX 77030.
6/1/24113

Table I. Mode of delivery and the incidence of peripartum blood transfusion

Delivery method	*No. of patients*	*Transfusions*	
		No.	*%*
Vaginal	1750	5	0.28
Primary cesarean	356	7	1.9
Repeat cesarean	150	1	0.66
TOTAL	2265	13	0.57

Material and methods

The records of all patients who were delivered and required blood transfusions at the University of California San Diego Medical Center from July 1 through

Table II. Details of the 13 patients who required peripartum blood transfusion

Estimated gestational age (wk)	*Delivery method*	*Hematocrit before transfusion (%)*	*Units of blood transfused*	*Risk factor*	*Comments*
32	Primary cesarean section	18.9	2	None	Breech
40	Primary cesarean section	18.9	2	None	Dystocia
30	Primary cesarean section	19.0	2	Placenta previa	Hysterectomy
38	Vacuum	20.6	5	None	Fetal distress
40	Vaginal	27.0	2	None	Uterine atony
29	Primary classical cesarean section	24.4	2	Placenta previa	Admission hematocrit 31%
24	Vaginal	24.8	2	None	Severe preeclampsia
40	Primary cesarean section	24.6	18	None	Hemolytic uterine syndrome
24	Primary classical cesarean section	17.0	2	None	Coagulopathy
38	Vacuum	20.7	9	None	Cervical laceration
38	Primary cesarean section	18.4	2	None	Bladder flap hematoma
37	Vaginal	17.9	2	Twins	Admission hematocrit 25%
40	Repeat cesarean section	20.7	1	Repeat cesarean section	Donor-specific blood

Dec. 31, 1988 were reviewed. Pertinent clinical data included maternal age, parity, source of prenatal care, gestational age at delivery, mode of delivery, estimated blood loss at delivery, hematocrit level before and after transfusion, and the number of units of packed red blood cells transfused. In addition, risk factors for peripartum transfusion were evaluated, including a history of postpartum hemorrhage, anemia, scheduled cesarean section, placenta previa, grandmultiparity, and multiple gestation. Patients were considered to have been ineligible for participation in an autologous donation program if their hematocrit level was <34%, estimated gestational age was <28 weeks, or if they had not received prenatal care.

Results

The incidence of blood transfusion during the study period was 13 in 2265 cases (0.57%). Transfusions were given in five of 1750 (0.28%) vaginal deliveries, 7 of 356 (1.9%) primary cesarean sections, and 1 of 150 (0.66%) repeat cesarean sections (Table I). The details of the thirteen patients undergoing transfusion of packed red blood cells are outlined in Table II. Twelve of the 13 (92%) patients required more than one unit of red blood cells. The mean (±SE) hematocrit before the transfusion was 21.0% ± 3.0% and the mean hematocrit after the transfusion was 27.6% ± 4.7%.

Of the 251 patients with identifiable risk factors for postpartum hemorrhage, 4 (1.6%) required transfusion. One of the 150 women (0.7%) with repeat cesarean sections, 1 of 27 (3.7%) multiple gestations, and two of eight (25%) patients with placenta previa were transfused. None of the 66 grandmultiparous women required blood transfusion (Table III).

Only two of the patients with identifiable high-risk factors (patients 3 and 13) would have been candidates for participation in an autologous blood donation program. The presence of a hematocrit <34% would have excluded both patients 6 (placenta previa) and 12 (twin gestation).[6]

Comment

The interest in autologous blood donation before elective operation is in large part a result of concern over the transmission of infectious disease. As the number of institutions offering such programs increases, the safety of the practice has been well documented. In a study of >300 high-risk patients who predeposited blood before elective surgery, only 4% experienced minor reactions and there were no serious adverse reactions.[7] Similarly, the safety of phlebotomy for predepositing of blood in the obstetric patient has been shown to be safe for mother and for fetus. Kruskall and Leonard[5] performed continuous fetal heart rate

monitoring during 61 donations and demonstrated reactive patterns throughout the procedure. The incidence of vasovagal reactions during phlebotomy has been shown to be similar for the pregnant and nonpregnant patient.[4]

The overall incidence of blood transfusions in our obstetric service of 0.28% for vaginal deliveries and 1.6% for cesarean sections is lower than that reported by other authors. Palmer et al.[8] cited a 0.3% to 1.6% rate of transfusion after vaginal deliveries and 4.6% to 7.3% after cesarean sections. Among the general obstetric population, Kruskall and Leonard[5] found that 1.2% of vaginal delivery patients and 2.2% of those having cesarean sections required transfusion. Our lower frequency of blood transfusion may reflect a greater tolerance of lower hematocrit values in the postpartum patient.

Rates of transfusion among patients who have banked their own blood range from 2.7% to 7.7%.[5, 9] Reports of higher transfusion rates are often associated with the liberal use of blood for hematocrit values that would be tolerated in the general obstetric population. In a study of 30 patients who participated in an autologous donation program, 15 (50%) had transfusions in the postpartum period. The authors[3] noted that patients who received their own blood had hematocrit values between 28% and 32%, whereas those who received homologous blood were symptomatic with hematocrits <27%. McVay et al.[4] showed that the overall rate of transfusion in their patients who participated in the donation program was 11%, yet only 4.3% had hemoglobin values <8 gm/dl before transfusion.

Our data suggest that the commonly accepted risk factors for postpartum hemorrhage and transfusion requirements do not permit clinicians to identify which patients will require blood transfusion. Fewer than 2% of the patients commonly targeted for participation in autologous donation programs (scheduled repeat cesarean section, placenta previa, multiple gestation, and grandmultiparous women) eventually required transfusion. Thus an attempt to use specific risk factors to select patients likely to need autologous blood would not have been accurate in our study population.

Our data show that the transfusion of a single unit of packed red blood cells is rare; 92% of our patients required multiple units. Because of the lower hematocrit values among obstetric patients and the tendency to restrict phlebotomies to the third trimester of pregnancy, it may be difficult to obtain multiple units per patient. Only 57% of the patients in the group of Herbert et al.[3] were able to donate more than one unit of blood. Thus is is likely that homologous blood would be required in addition to autologous transfusions.

Table III. Incidence of blood transfusion in patients with risk factors for postpartum hemorrhage

Risk factor	*No. of patients*	*Transfusions*	
		No.	*%*
Repeat cesarean section	150	1	0.7
Multiple gestation	27	1	3.7
Placenta previa	8	2	25
Grandmultiparity	66	0	
TOTAL	251	4	1.6

We believe, as has been suggested by others,[10] that careful restriction of blood products in an obstetric population is likely to reduce the frequency so that transfusion is rarely required. The inability to accurately predict which patients will require blood transfusion, combined with the low incidence of transfusions, suggests that the practice of autologous donation of blood among obstetric patients may not be justified.

REFERENCES

1. Autologous blood transfusions. Council on scientific affairs. JAMA 1986;256:2378-80.
2. Miles G, Browne WH, Barrick RG. Autologous transfusions for elective cesarean section. AM J OBSTET GYNECOL 1969;103:1166-7.
3. Herbert WN, Owen HG, Collins ML. Autologous blood storage in obstetrics. Obstet Gynecol 1988;72:166-70.
4. McVay PA, Hoag RW, Hoag SM, Toy PT. Safety and use of autologous blood donation during the third trimester of pregnancy. AM J OBSTET GYNECOL 1989;160:1479-86.
5. Kruskall MS, Lenoard S, Klapholz H. Autologous blood donation during pregnancy: analysis of safety and blood use. Obstet Gynecol 1987;70:938-40.
6. American Association of Blood Banks. Standards for blood banks and transfusion services. 11th ed. Arlington, Virginia: American Association of Blood Banks, 1984.
7. Mann M, Sachs HJ, Goldfinger D. Safety of autologous blood donation prior to elective surgery for a variety of potentially high-risk patients. Transfusion 1983;23:229-32.
8. Palmer RH, Kane J, Churchill WH, Goldman L, Komaroff AL. Cost and quality in the use of blood bank services for normal deliveries, cesarean sections and hysterectomies. JAMA 1986;256:219-23.
9. Druzin ML, Wolf CFW, Edersheim TG, Hutson JM, Kogut EA, Salamon N. Donation of blood by the pregnant patient for autologous transfusion. AM J OBSTET GYNECOL 1988;159:1023-27.
10. Simon TL. Postpartum blood requirements: should autologous donations be considered? [Letter]. JAMA 1988; 259:2021.

The Maylard incision in gynecologic surgery

B. Frederick Helmkamp, MD, and Hans-B. Krebs, MD
Falls Church, Virginia

The transverse muscle-splitting Maylard incision was used in 175 gynecologic patients who required pelvic-abdominal surgery. One hundred fifty-three patients (87%) had pelvic malignancy; other indications for operation included uterine myomas, endometriosis, tuboovarian abscess, and benign ovarian cysts. Exposure was excellent: 54% of patients underwent pelvic lymphadenectomy and 17% underwent paraaortic lymphadenectomy. Twelve patients (6.9%) had wound complications, but there was neither long-term morbidity nor mortality associated with the incision. Fifty-six patients (32%) received preoperative or postoperative pelvic radiation therapy with no detrimental effect on wound healing. The Maylard incision is cosmetic, strong, easily learned, and has an acceptable complication rate. Unless a vertical incision is indicated, the Maylard technique is preferred when optimal exposure and accessibility to the pelvis are required. (AM J OBSTET GYNECOL 1990;163:1554-7.)

Key words: Maylard incision, pelvic abdominal surgery

One of the most critical factors in successful surgery is adequate exposure. In 1907 Ernest Maylard, the British surgeon, advocated cutting the rectus muscles with a transverse abdominal incision.[1] This new technique greatly facilitated exposure within the abdomen and pelvis, especially when compared with the more commonly performed Pfannenstiel incision, which was initially described in 1900. Maylard also stressed two other factors in his departure from vertical incisions: a significant decrease in incisional hernia, and more rapid healing by secondary intention with improved cosmetic results if wound infection did occur. Subsequent articles emphasized the anatomic and physiologic advantages of the Maylard incision and its low complication rate.[2-5] In 1954 Tollefson and Russell[6] published data on the Maylard incision used in more than 600 gynecologic and obstetric procedures. Wound complications were minimal (5%), and only one of two patients in whom a hernia developed needed surgical correction. In 1981 Mann et al.[7] reported their experience with the Maylard incision in 168 patients who had radical hysterectomy.

This article reports a prospective study on the Maylard incision in a large series of gynecologic patients. Our modifications of the technique previously reported by Maylard and by Tollefson and Russell are presented in detail to provide the pelvic surgeon with increased confidence in use of the Maylard incision.

From the Department of Obstetrics and Gynecology, Fairfax Hospital. Received for publication February 12, 1990; revised July 12, 1990; accepted July 20, 1990.
Reprint requests to: B. Frederick Helmkamp, MD, 3289 Woodburn Road, Suite 320, Annandale, VA 22003.
6/1/24035

Patients and methods

One hundred seventy-five patients underwent pelvic-abdominal operations through a Maylard incision between March 1982 and September 1989. All procedures were performed by the house staff under our supervision at Fairfax Hospital (George Washington University Medical Center), Strong Memorial Hospital (University of Rochester), and the Medical College of Virginia. Only patients with an incision above the symphysis pubis were included in this study; we have previously reported our experience with the transverse periumbilical incision.[8] Study patients ranged in age from 21 to 80 years (mean, 46 years), and weights ranged from 44 to 180 kg (mean, 78 kg). The indications for operation are listed in Table I.

Thirty-three patients (19%) had preoperative external beam pelvic radiation therapy. Five patients had previous Pfannenstiel incisions, and two patients had large midline incisions with Marlex (C.R. Bard, Inc., North Billerica, Mass.) mesh repair of incisional hernias. Major medical problems (diabetes mellitus, cardiac disease, chronic obstructive pulmonary disease, and osteoporosis with vertebral fractures) were encountered in 20 patients (11.4%).

Surgical technique. A transverse skin incision is made 3 to 8 cm above the symphysis pubis and is carried down to the anterior rectus sheath. The exact location and length of the skin incision depend on the patient's age and weight, indication for operation, and previous abdominal or pelvic operation(s). The fascial sheath is then incised transversely to the lateral borders of the rectus muscles. By use of gentle finger dissection, the rectus muscle is teased away from the transversalis fascia, and a small right-angle retractor is placed beneath

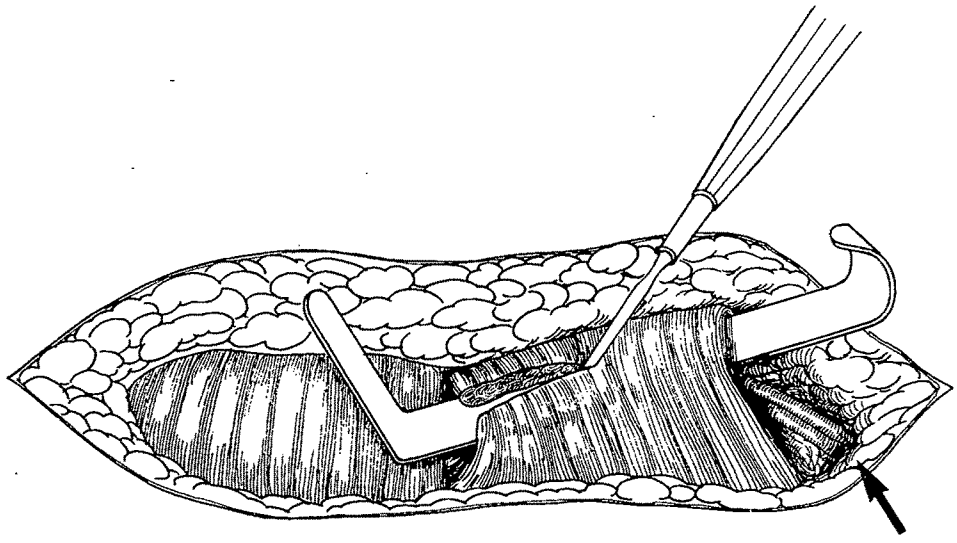


Fig. 1. A small right-angle retractor is used to elevate the rectus muscle and expose the inferior epigastric vessels *(arrow)*. Electrocautery is used to transect the rectus muscle.

it. The inferior epigastric vessels are found beneath the lateral one third of the muscle and are left within the transversalis fascia. The rectus muscle is then divided slowly by electrocautery (Fig. 1). Fine tonsil clamps are used to dissect the inferior epigastric bundle, and these vessels are tied with 2-0 silk. If the inferior epigastric vessels are readily visible and quite lateral in location, they may be ligated before the muscle is divided. The second rectus muscle is divided in a similar manner. Beginning laterally, the transversalis fascia and peritoneum are divided transversely with electrocautery or curved Mayo scissors.

A layered closure is readily accomplished. The peritoneum and transversalis fascia are closed with a continuous running 2-0 synthetic absorbable suture. A 10 mm flat Jackson-Pratt drain is placed across this suture line in the subfascial space, and exits through a separate stab incision (Fig. 2). It is removed when drainage is less than 40 ml per 24-hour period. The anterior rectus sheath is then closed with either interrupted or continuous suture of 0 synthetic absorbable or nonabsorbable material. No attempt is made to suture the retracted rectus muscles or include them in the fascial closure. The subcutaneous tissue is copiously irrigated with saline solution, and the skin edges are approximated with staples or a subcuticular suture.

Results

The surgical procedures performed in this series of 175 patients are listed in Table II. Thirty patients (17%) had a paraaortic node dissection as part of their operation.[9]

Table I. Preoperative indications for operation

Diagnosis	*No. of patients*
Cervical carcinoma-sarcoma	91
Endometrial carcinoma	58
Endometrial hyperplasia	4
Uterine leiomyomas–endometriosis	12
Benign tumors of ovary	4
Tuboovarian abscess	2
Hydrosalpinx*	1
Ovarian carcinoma	1
Vaginal clear cell carcinoma	1
Vulvar carcinoma	1
TOTAL	175

*Histologic examination confirmed fallopian tube carcinoma.

All patients had closed suction drainage of the subfascial space. The drain was left in place from 2 to 6 days (mean, 3.2 days) and collected from 24 to 350 ml fluid (mean, 120 ml) (one patient was excluded; see the paragraph on complications).

Fifty-six patients (32%) in this series received whole-pelvis radiation therapy. Thirty-three patients (19%) had preoperative radiation therapy, whereas 23 patients (13%) required postoperative radiation therapy.

Complications occurred in 12 patients (6.9%) and are summarized in Table III. Of the six patients with superficial wound infection, five were markedly obese (111 to 159 kg), three had insulin-dependent diabetes mellitus, and two had preoperative radiation therapy. The first four patients were treated with wound packing and long-term healing by secondary intention,

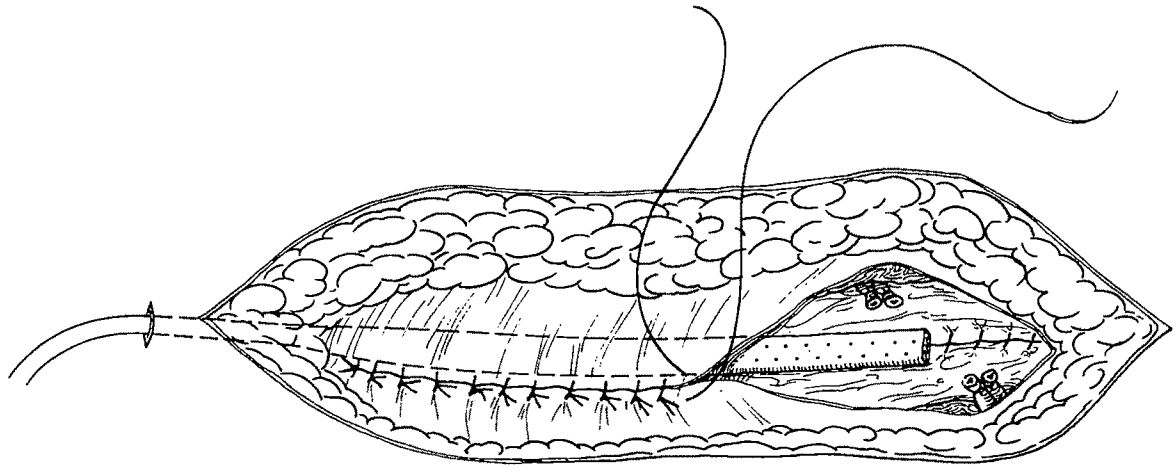


Fig. 2. The anterior fascia is closed over a 10 mm flat Jackson-Pratt drain.

Table II. Surgical procedures

Operation	*No. of patients*
Hysterectomy, with or without salpingo-oophorectomy	73
Hysterectomy, with or without salpingo-oophorectomy, pelvic lymphadenectomy	29
Radical hysterectomy, with or without salpingo-oophorectomy, pelvic lymphadenectomy*	59
Radical hysterectomy, paraaortic and pelvic lymphadenectomy, vaginectomy	1
Paraaortic and pelvic lymphadenectomy	6
Salpingo-oophorectomy	6
Reassessment laparotomy†	1
TOTAL	175

*Twenty-three of these patients also had paraaortic lymphadenectomy.

†Patient only agreed to operation through previous Pfannenstiel incision.

Table III. Postoperative complications

Complications	*No. of patients*
Superficial wound infection	6
Fasciitis	4
Subfascial hematoma	2

whereas the last two patients underwent rapid secondary closure with antibiotic administration.[10] Four patients had fasciitis that necessitated radical wound debridement and long-term healing by secondary intention. Three of these patients were obese (90, 91, and 160 kg) and had insulin-dependent diabetes mellitus. The two patients with subfascial hematoma had abnormal clotting factors but received minidose heparin. One patient was managed conservatively with prolonged catheter drainage (1090 ml over 11 days), whereas the second patient required evacuation of the hematoma on the second postoperative day.

Incisional hernia did not occur in any of the patients, including the four patients with fasciitis who required radical wound debridement of the anterior fascia. There were no deaths in this series.

Comment

The transverse abdominal incision has been used for over 150 years, and "... is based on sound anatomic, physiologic, and clinical principles."[2] When compared with a vertical incision, the following advantages of a transverse incision are readily noted: (1) a virtual absence of postoperative dehiscence[2, 6, 11, 12] and incisional hernia,[1, 2, 6, 12] (2) a significant decrease in pulmonary complications and postoperative discomfort,[2, 4, 6, 13] and (3) excellent cosmetic results.[1, 4] Conversely, limited access to the pelvis and upper abdomen has always been the primary disadvantage of the Pfannenstiel incision, the most popular transverse incision used by pelvic surgeons. Fortunately, Ernest Maylard and his proponents have resolved this dilemma with the transverse muscle-splitting incision. Although initial concern was raised with regard to the healing of the abdominal wall with the Maylard incision, numerous clinical and animal studies[1, 3, 4, 6, 12, 13] have shown that fibroblastic proliferation creates a new transverse band of fibrous tissue (linea transversa) where the muscle is cut.

Our series of 175 patients illustrates the adaptability of the Maylard incision depending on the indications for operation, the anticipated surgical procedure, the patient's age, weight, and previous surgical history, and known medical problems. For example, we often use

the Maylard incision in adolescents and young women with ovarian neoplasms when the index of suspicion for malignancy is low. Eighty-seven percent of all procedures in this series were performed for pelvic malignancies. We concur with Mann et al.[7] that the Maylard incision is preferred for radical hysterectomy. The excellent exposure obtained with the Maylard incision is emphasized by the fact that 95 patients (54%) underwent pelvic lymphadenectomy, and 30 patients (17%) also had paraaortic lymph node dissection.

Two modifications in the technique popularized by Maylard and Tollefson and Russell are suggested. First, the rich anastomotic blood supply to the rectus muscle allows the inferior epigastric vessels to be routinely ligated. This will minimize tearing of these vessels during lateral retraction and maximize the incision through the transversalis fascia and peritoneum. Ligation of these vessels was not described by Maylard,[1] and was performed in only one third of the patients of Tollefson and Russell.[6] Second, a closed suction drain is placed in the subfascial space in all patients. Our average collection of 120 ml drainage supports its routine use to minimize hematoma or seroma formation. This is in contrast to the series by Mann et al.[7] in which drains were used in only 44% of patients.

Our complication rate of 6.9% is comparable with the 4% to 5% rate reported by Mann et al.[7] and Tollefson and Russell.[6] Marked obesity and diabetes are recognized factors in poor wound healing and were present in the majority of the 10 patients in whom superficial wound infection or fasciitis developed. We believe that the two cases of subfascial hematoma were probably preventable if strict attention had been paid to coagulation values.

Femoral neuropathy and other nerve injuries have been reported in patients with low transverse incisions.[14, 15] Neither these injuries nor incisional hernia and dehiscence were encountered in this series.

There are few disadvantages to the Maylard incision. Although a few more minutes are required for transection of the muscles and ligation of the inferior epigastric vessels,[16] we agree with Christopherson[12] that the Maylard incision should not be used if prompt visualization of the abdomen or pelvis is a critical factor. Second, a vertical incision is preferred when optimal exposure of the upper abdomen is required, for example, for exploration for ovarian carcinoma or other advanced pelvic malignancies. Conversely, we disagree with the conclusion by Mann et al.[7] that the Maylard incision should not be used in a pelvis that has been treated by radiation. Fifty-six patients in this series received pelvic radiation therapy, and only two of these were among the 10 patients in whom wound complications developed. Our data show that preoperative radiation therapy is not a contraindication for use of the Maylard incision, and that wound healing is not adversely affected by postoperative radiation therapy.

As shown in this series of 175 patients, the transverse muscle-splitting incision is adaptable to many different clinical situations. It is easily learned, well accepted by patients, and has a low complication rate. When optimal pelvic exposure is necessary, and speed of entry and access to the upper abdomen are not critical factors, the Maylard incision is our preferred surgical incision.

REFERENCES

1. Maylard AE. Direction of abdominal incisions. Br Med J 1907;2:895-901.
2. Rees VL, Coller FA. Anatomic and clinical study of the transverse abdominal incision. Arch Surg 1943;47:136-46.
3. Rosenblatt MS, Colver D. Transverse upper abdominal incisions. Surg Gynecol Obstet 1945;80:641-2.
4. Pernworth P. The transverse incision in abdominal surgery. Am J Surg 1946;72:573-5.
5. Gurd FN. Fundamentals of technique in the transverse abdominal incision. Surgery 1946;20:217-23.
6. Tollefson DG, Russell KP. The transverse incision in pelvic surgery. Am J Obstet Gynecol 1954;68:410-22.
7. Mann WJ Jr, Orr JW Jr, Shingleton HM, et al. Perioperative influences on infectious morbidity in radical hysterectomy. Gynecol Oncol 1981;11:207-12.
8. Krebs H-B, Helmkamp BF. Transverse periumbilical incisions in the massively obese patient. Obstet Gynecol 1984;63:241-5.
9. Belinson JL, Goldberg MI, Averette HE. Para-aortic lymphadenectomy in gynecologic cancer. Gynecol Oncol 1979;7:188-91.
10. Gottrup F, Gjode P, Lundhus F, Andrup H, Holm CN, Terpling S. Management of severe incisional abscesses following laparotomy. Arch Surg 1989;124:702-4.
11. Helmkamp BF. Abdominal wound dehiscence. Am J Obstet Gynecol 1977;128:803-7.
12. Christopherson WA. Abdominal incisions and their anatomic basis. Contemp Ob/Gyn 1988;32:38-56.
13. Daversa B, Landers D. Physiologic advantages of the transverse incision in gynecology. Obstet Gynecol 1961; 17:305-10.
14. Hoffman MS, Roberts WS, Cavanagh D. Neuropathies associated with radical pelvic surgery for gynecologic cancer. Gynecol Oncol 1988;31:462-6.
15. Goldman JA, Feldberg O, Dicker D, et al. Femoral neuropathy subsequent to abdominal hysterectomy. A comparative study. Eur J Obstet Gynecol Reprod Biol 1985; 20:385-92.
16. Ayers JWT, Morley GW. Surgical incision for cesarean section. Obstet Gynecol 1987;70:706-8.

Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level

Eric Jauniaux, MD,* Donald Gibb, MD, Gonzalo Moscoso, MD, and Stuart Campbell, FRCOG

London, England

A placental lesion associated with elevated maternal serum α-fetoprotein level and a positive Kleihauer-Betke test is reported. Serial ultrasonograms showed a progressive increase in the echogenicity of the lesion that corresponded to a large intervillous thrombosis after delivery. This case illustrates the importance of detailed ultrasonographic and pathologic placental investigations in pregnancies with unexplained elevated levels of α-fetoprotein. (Am J Obstet Gynecol 1990;163:1558-60.)

Key words: Prenatal diagnosis, α-fetoprotein, placenta, ultrasonography

From the Obstetric Ultrasound Unit, Department of Obstetrics and Gynecology, and the Fetal and Perinatal Pathology Unit, King's College Hospital School of Medicine and Dentistry, University of London.
Received for publication February 8, 1990; revised May 4, 1990; accepted May 18, 1990.
Reprint requests: Eric Jauniaux, MD, Department of Obstetrics and Gynecology, Academic Hospital Erasme, 808 Route de Lennik, 1070 Brussels, Belgium.
**Research fellow from the Free University of Brussels (ULB), Belgium, supported by the British Council (Sir Alexander Fleming Award, 1989).*
6/1/22485

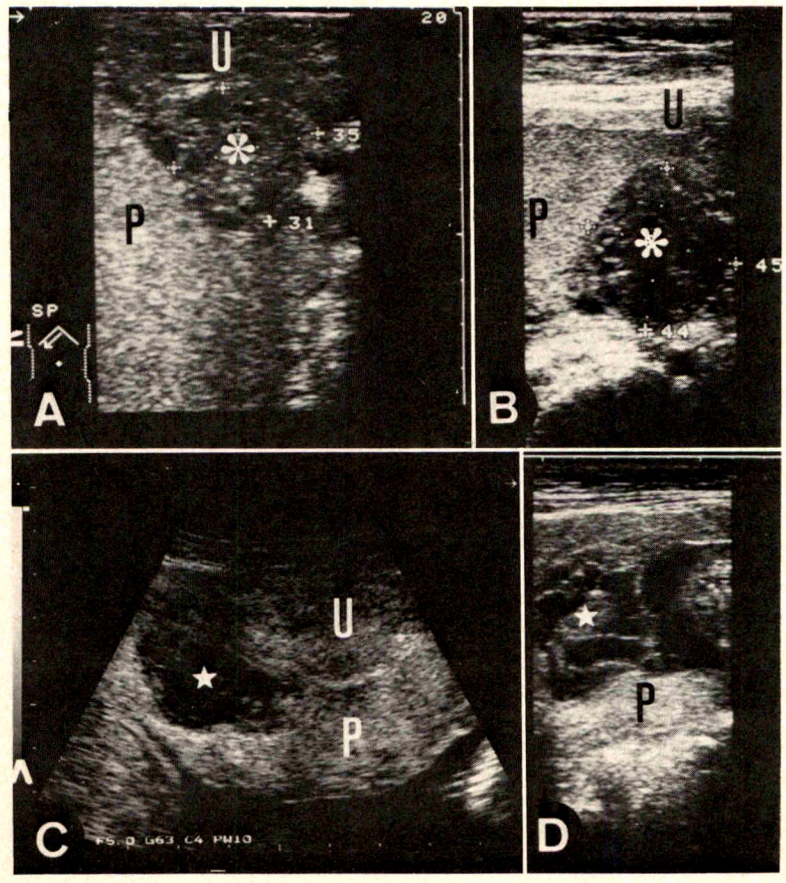


Fig. 1. Composite compound scan of uterus *(U)* and placenta *(P)* shows heterogeneous and hypoechoic placental lesion *(asterisk)* at 17 **(A)** and 22 **(B)** weeks' gestation. For comparison, **C,** a retroplacental hematoma *(star)* at 32 weeks, 2 weeks after an invasive procedure, and **D,** a recent marginal hematoma *(star)* at 18 weeks.

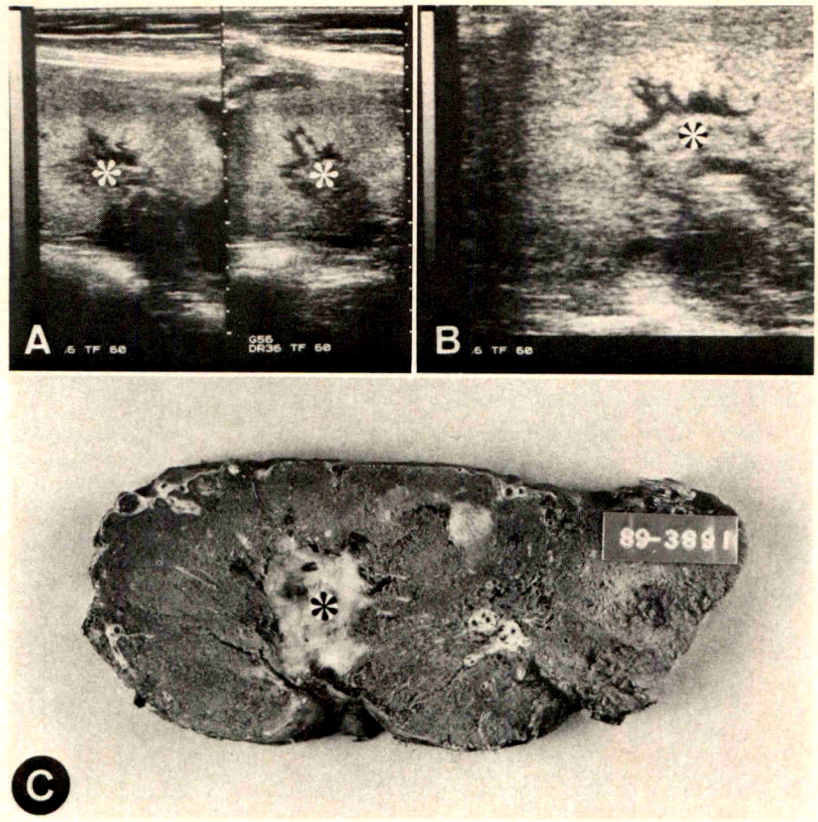


Fig. 2. A, Transverse and longitudinal ultrasonogram of placenta shows placental lesion at 35 weeks *(asterisk)*. **B,** Lesion is isoechoic and surrounded by sonolucent spaces containing turbulent blood flow on real-time imaging. **C,** Macroscopic view of lesion *(asterisk)* corresponds histologically to an old intervillous thrombosis.

Maternal serum α-fetoprotein (AFP) concentrations are routinely performed between 16 and 20 weeks' gestation as a screening test for different fetal anomalies. Elevated AFP levels can also be found in association with well-defined placental lesions without associated fetal anomalies such as chorioangiomas[1] or in combined fetoplacental pathologic conditions such as triploidy.[2] We described an unusual placental lesion that ultrasonographically resembled a hematoma or a chorioangioma and was associated with an elevated maternal serum AFP level.

Case report

A 29-year-old healthy nonsmoking primigravid woman was referred because of elevated maternal serum AFP levels (4.5 and 4.8 multiples of the median). At 18 weeks' gestation ultrasonography showed a singleton and anatomically normal fetus with measurements consistent with the estimated gestation. The placenta was fundal and contained a 3.1 × 3.5 cm heterogeneous and hypoechoic lesion (Fig. 1, *A*). A hematoma or a chorioangioma was suspected and ultrasonographic follow-up of the pregnancy was started. At that time a Kleihauer-Betke test had positive results.

At 22 weeks the lesion was larger, well demarcated (Fig. 1, *B*), and contained turbulent blood flow on real-time scanning. Weak flow velocity waveforms with maternal characteristics were detected at the level of the lesion on color Doppler flow imaging. These findings were compared with the ultrasonographic features of a retroplacental hematoma and of a subchorionic (marginal) hematoma, both of which were subsequently confirmed after delivery. In these two cases no blood flow was found in the lesion. These lesions were hyperechoic to isoechoic in the acute stage (Fig. 1, *D*) and hypoechoic to sonolucent within 2 weeks (Fig. 1, *C*).

At 29 weeks slow fetal growth (5th percentile) and reduced amniotic fluid volume were noted. Fetal and uteroplacental Doppler studies were within our normal ranges. The placental lesion was homogeneous, isoechoic (Fig. 2, *A*), and surrounded by small sonolucent spaces (Fig. 2, *B*) that contained turbulent flow. Similar antepartum features were observed until term. Because of breech presentation and poor fetal growth, the patient was delivered by cesarean section of a healthy female infant weighing 2230 gm. The postpartum course was uneventful.

The placenta weighed 410 gm. Examination of the slices showed a white lesion of 3 × 3 × 4 cm, composed histologically of laminated fibrin surrounded by several groups of infarcted villi (Fig. 2, *C*). There was no evidence of a retroplacental hematoma.

Comment

Ultrasonographic investigation of the placenta in patients with elevated maternal serum AFP levels has shown a high incidence of retroplacental or subchorionic placental hemorrhage.[1] However, in this study serial ultrasonographic examination and pathologic correlation were not performed.

The placenta is a dynamic organ. As demonstrated in this case, the ultrasonographic appearances are dependent on gestational age and the evolution of the lesion. At the time of AFP screening, the lesion was hypoechoic and contained turbulent blood flow. Six weeks later the lesion was more echogenic. In contrast, resolving hematomas become sonolucent and do not demonstrate blood flow. A placental chorioangioma was also unlikely because color Doppler flow studies of the lesion only detected maternal flow velocity waveforms.

The association of an increased maternal serum AFP level with a positive Kleihauer-Betke test is suggestive of a breakdown of the placental barrier[2] and should lead to detailed assessment of placental appearance.

Elevated maternal serum AFP levels, combined with abnormal placental ultrasonographic features, may be an early indication of subsequent perinatal complications, particularly fetal intrauterine growth retardation. Further placental ultrasonographic and pathologic investigations in a prospective manner are needed to validate the relationship between the development of placental lesion, elevated maternal serum AFP, and fetal outcome.

REFERENCES

1. Fleischer AC, Kurtz AB, Wapner RJ, et al. Elevated alpha-fetoprotein and a normal fetal sonogram: association with placental abnormalities. AJR 1988;150:881-3.
2. Berkeley AS, Killackey MA, Cederqvist LL. Elevated maternal serum α-fetoprotein levels associated with breakdown in fetal-maternal-placental barrier. AM J OBSTET GYNECOL 1983;146:859-61.

Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes

Alistair B. Roberts, MD, and Jennifer M. Mitchell, DMU
Auckland, New Zealand

Fetal lung length was measured directly with ultrasonography in 20 patients with prolonged rupture of membranes, commencing before 25 weeks' gestation. Measurements were made weekly and compared with data collected from 310 normal pregnancies. Measurement of fetal lung length by ultrasonography was a good predictor of pulmonary hypoplasia, predicting >90% of cases. There was a good correlation between lung size assessed by the last ultrasonographic examination and lung weight postmortem ($r = 0.783$, $p < 0.05$). Lung length measurements were superior to fetal chest circumference measurements in the identification of pulmonary hypoplasia. There was a significant negative association between the amount of amniotic fluid and pulmonary hypoplasia ($p < 0.05$). There were fetuses with pulmonary hypoplasia that had respiratory movements seen at the majority of ultrasonographic examinations. (AM J OBSTET GYNECOL 1990;163:1560-6.)

Key words: Fetal lung, fetal breathing, oligohydramnios, pulmonary hypoplasia, ultrasonography

From the Department of Obstetrics, Auckland University Medical School, National Women's Hospital.
Received for publication January 31, 1990; revised May 18, 1990; accepted July 27, 1990.
Reprint requests: A. B. Roberts, MD, Department of Obstetrics and Gynecology, Auckland University Medical School, National Women's Hospital, Claude Road, Auckland, New Zealand.
6/1/24145

Fetal pulmonary hypoplasia is a significant contributor to neonatal death.[1] It occurs as a consequence of prolonged rupture of membranes,[2] renal agenesis, diaphragmatic hernia, pleural effusions, and skeletal dysplasias.[3-5] Antepartum prediction of pulmonary hypoplasia has been elusive.[6]

Ultrasonography has contributed by use of mea-

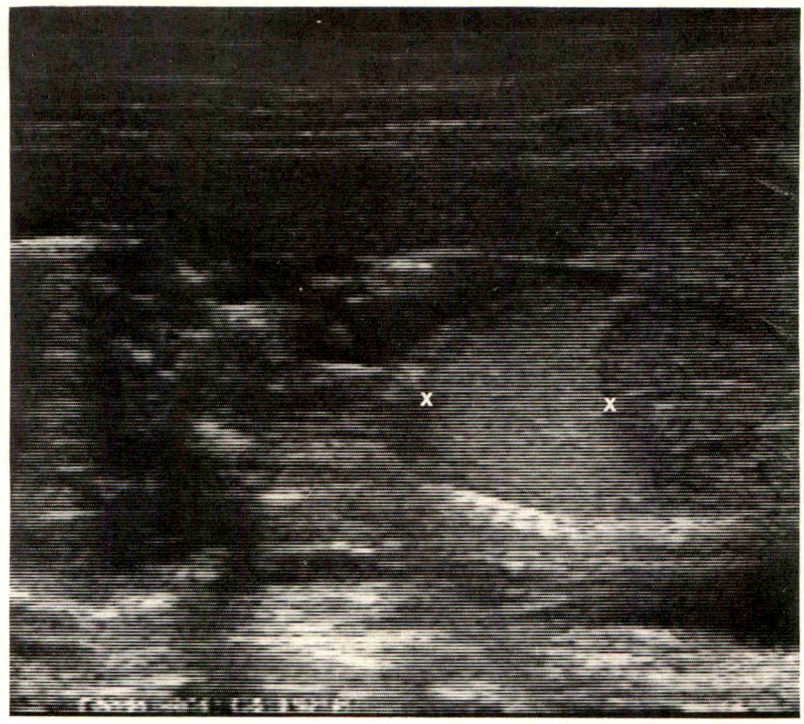


Fig. 1. Ultrasonogram of the fetal lung in sagittal section with *crosses* indicating method of measurement from base of lung at dome of diaphragm to apex of lung adjacent to clavicle.

surements of chest circumference and area,[7] either directly or by comparison with other fetal measurements,[8, 9] but no direct measurements of lung size have been reported. Attempts have been made to predict hypoplasia by use of fetal breathing movements but the results have been contradictory.[10, 11]

In this study we aimed to measure the fetal lung directly with ultrasonography. We elected to measure fetal lung length and use this as an index of lung size. We first studied a group of patients with normal pregnancies and then a group of patients with prolonged rupture of membranes. In the latter group recordings were also made of liquor volume and breathing movements.

Patients and methods

A total of 310 uncomplicated pregnancies between 12 and 40 weeks' gestation were studied. All patients had regular menstrual periods and a confirmatory ultrasonographic examination before 20 weeks' gestation. They were all singleton pregnancies and were delivered within 2 weeks of the expected date of delivery. Growth-retarded infants below the 5th percentile and macrosomic infants above the 95th percentile were excluded (size was compared with nomograms from National Women's Hospital). The data collected were cross-sectional with one measurement per patient. All data were collected as part of normal ultrasonographic examinations requested by each patient's physician. Examinations were performed with Toshiba real-time ultrasonographic equipment. Measurements were made of fetal lung length and fetal chest circumference. Great care was taken to ensure that there were adequate data points at each stage of gestation.

The length of the fetal lung was measured by taking a sagittal section through the fetal chest (Fig. 1). The lung was measured from the tip of the apex to the base of the lung on the dome of the diaphragm during fetal apnea. Data collected from the left lung in an initial pilot study on 10 patients showed no differences between the left and right lungs. Care was taken to ensure that the apex was reached and also to identify the top of the diaphragm because the diaphragmatic insertions are significantly lower. The clavicle was used as a marker to ensure that the apex was reached. The decision to use the left lung was an arbitrary one to ensure that the same lung was always measured. The left lung was always measured including the cases with ruptured membranes. We have since realized that the right lung is easier to visualize because there is less encroachment from the heart.

Chest circumference was measured in a plane at right angles to the fetal spine with atrioventricular valves as the marker. This approximates a four-chamber view of the fetal heart but was slightly rotated to ensure that the section was at right angles to the spine.

Table I. Data from 20 patients with ruptured membranes

Patient No.	*Gestation at rupture (wk)*	*No. of weeks ruptured**	*Last ultrasonogram† (wk)*	*Lung length‡ (mm)*	*Breathing observed§*	*Amniotic fluid average‖ (cm)*	*Pulmonary hypoplasia*	*Status*
1	22	1	23	23	2/2	1.4	No	Dead
2	18	4	21	13	0/1	0	Yes	Dead
3	20	11	30	23	3/6	1.5	Yes	Dead
4	23	1	23	27	1/1	1.0	No	Dead
5	21	2	23	18	0/2	1.0	Yes	Dead
6	18	12	30	23	6/7	1.2	Yes	Dead
7	24	3	27	24	2/2	0.5	Yes	Dead
8	22	3	25	22	1/2	0.5	Yes	Dead
9	23	3	26	29	2/2	1.8	No	Alive
10	24	5	28	32	3/3	1.2	No	Alive
11	22	3	23	21	0/2	0	Yes	Dead
12	21	4	24	24	3/4	0.8	Yes	Dead
13	24	2	26	28	2/2	2.3	No	Alive
14	24	10	34	54	4/4	3.7	No	Alive
15	21	5	26	22	0/2	0	Yes	Dead
16	22	3	22	20	0/1	0	Yes	Dead
17	23	3	25	21	2/2	0.5	Yes	Dead
18	23	1	23	19	1/2	0.5	Yes	Dead
19	24	1	25	26	1/1	2.0	No	Alive
20	24	1	24	30	0/2	1.0	No	Alive

*Number of weeks that membranes were ruptured before delivery.
†Gestational age when last ultrasonographic examination was performed.
‡Lung length measurement at last ultrasonographic examination.
§Ratio of examinations during which breathing was observed/total number of examinations.
‖Mean amniotic fluid pocket size over all examinations.

Patients with ruptured membranes before 25 weeks' gestation were also studied. Drainage of amniotic fluid was confirmed clinically by examination and fluid continued to drain until delivery in all cases. In no case was there clinical evidence of amniotic infection; there were no significant contractions until just before delivery. No tocolytic agent was given in any case, and all patients elected conservative treatment. They all had ruptured membranes of >1 weeks' gestation and full details are given in Table I. Fetal lung measurements were made once a week (left lung), and estimates were made of the amount of amniotic fluid and whether fetal breathing movements were present. Fetal breathing was defined as any chest wall or diaphragmatic movement that had the appearance of breathing and was not associated with other fetal movements. At least two breaths within 6 seconds had to occur before breathing was recorded. One isolated breath was not considered "breathing." The number of examinations during which breathing was seen were recorded. The mean examination time was approximately 20 minutes. The amount of amniotic fluid was assessed by measurement of the largest clear vertical pocket of fluid on each occasion. The mean size of these pockets was calculated for all examinations.

If fetal or neonatal death occurred only patients with postmortem evidence of lung size or strong clinical and radiologic evidence were included. The pathologic diagnosis of pulmonary hypoplasia was made with the ratio of lung weight/body weight. Ratios <0.012 were accepted as abnormal.[12]

Scattergrams of lung length and chest circumference against gestational age were drawn and regression analysis used with estimation of the regression line with 95% prediction intervals.

Reproducibility of the lung length measurement and chest circumference was assessed between different observers measuring "blind" to each other's results, and for the same observer. Interobserver differences of lung length were <8% for 95% of the time. Intraobserver differences were <6%. Chest circumference values were 12% and 8% for inter- and intraobserver differences, respectively. These were not influenced significantly by gestational age. Reproducibility studies were not performed separately in patients with ruptured membranes. Statistical analysis of data from patients with ruptured membranes was made with the Epistat (Epistat Services, Richardson, Tex.) computer program with Fisher's exact test, Pearson's correlation coefficent, and multiple logistic regression analysis. Regression equations for lung length and chest circumference data were analyzed and produced by the Mathematics Department at Auckland University.

Results

Nomograms for fetal lung length and chest circumference with respect to gestational age were produced. The regression equation for lung length was

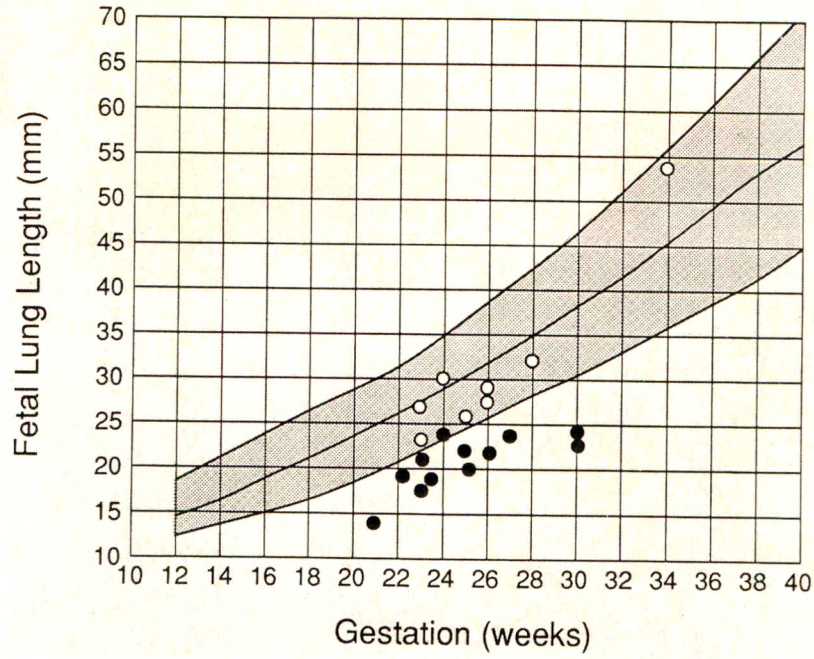


Fig. 2. Mean + 95% prediction intervals for normal fetal lung length plotted against gestational age. *Superimposed circles* represent lung length measurements in patients with ruptured membranes. *Open circles* are those with normally grown lungs; *closed circles* are those with pulmonary hypoplasia.

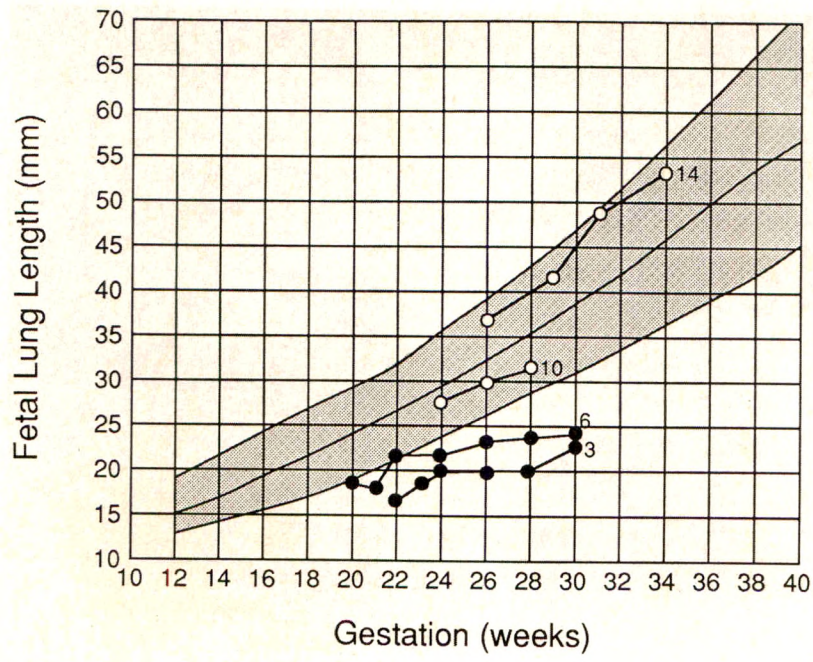


Fig. 3. Normal graph for lung length with *superimposed circles* representing patients with ruptured membranes of >3 weeks. *Open circles* represent patients in whom fetus and neonate had normally grown lungs; *closed circles* represent neonates with pulmonary hypoplasia.

as follows: Log lung length = 1.7528 + 0.0802 × gestational age − 0.00058 × gestational age^2.

The scattergram of lung length against gestational age is shown in Fig. 2.

A total of 20 patients with prolonged ruptured membranes were eventually included in the study. All had ruptured membranes of ≥1 week with a range of 1 to 12 weeks (mean, 3.9 weeks). Membranes ruptured between 18 and 24 weeks' gestation (mean, 22.2 weeks). There were six survivors. Of the 14 deaths 12 had

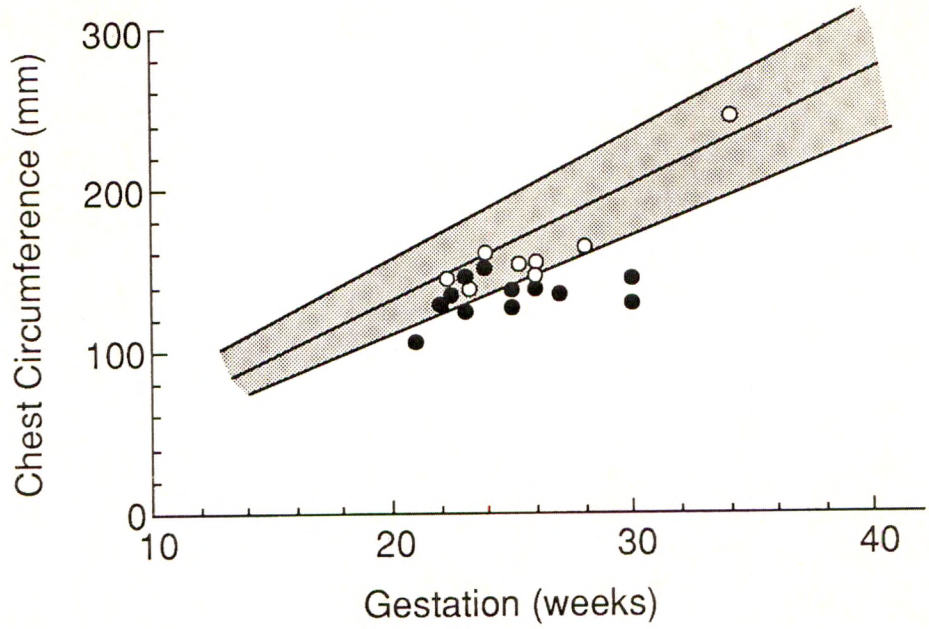


Fig. 4. Mean + 95% prediction intervals for fetal chest circumference plotted against gestational age. *Superimposed circles* represent measurements made in patients with ruptured membranes. *Open circles* represent normal lungs; *closed circles* represent pulmonary hypoplasia.

evidence of pulmonary hypoplasia (10 postmortem and two clinical and radiologic) (Table I).

There was a significant association between ultrasonographic measurement of lung length and pulmonary hypoplasia ($p < 0.001$). Of the 12 fetuses with pulmonary hypoplasia, 11 had a last ultrasonographic measurement of lung length that was outside the 95% prediction intervals (Fig. 2). The results of lung growth in four fetuses with ruptured membranes >4 weeks are plotted in Fig. 3. Lung size by last ultrasonographic examination correlated well with lung weight postmortem ($r = 0.783$, $p < 0.05$).

Chest circumference measurements were below the 95% prediction intervals in eight of the 12 fetuses with pulmonary hypoplasia (Fig. 4).

Average size of amniotic fluid pockets ranged from 0 to 3.7 cm, with a mean of 1.05. Fetuses with amniotic fluid <1.5 cm were more likely to have lung hypoplasia ($p < 0.05$). There were four fetuses with no amniotic fluid seen and in all instances the fetuses had pulmonary hypoplasia. The four fetuses with pockets of fluid >1.5 cm had normal lung growth (Table I).

Breathing movements were observed in 63% of examinations. There was no significant association between the presence or absence of breathing and lung hypoplasia. However, fetuses that were breathing during the majority of examinations (>50%) were significantly more likely to have normal lung growth ($p > 0.05$). If membranes ruptured before 22 weeks' gestation then pulmonary hypoplasia was more likely ($p < 0.05$).

In 10 of 12 fetuses with pulmonary hypoplasia the membranes were ruptured for ≥3 weeks, but the association between duration of ruptured membranes and pulmonary hypoplasia did not reach statistical significance.

Multiple logistic regression analysis was used with the computer selecting the variables that contribute to the prediction of pulmonary hypoplasia. A significance level of 0.05 was chosen for inclusion. Lung length, amount of amniotic fluid, gestational age at rupture, and average time spent breathing were selected to enter the regression in that order, with p values of 0.000002, 0.0002, 0.001, 0.02, respectively. Lung length alone accounted for 84.3% of the log likelihood. The addition of either of the other variables could be used to correctly classify 100% of cases. For example a model with lung length and amount of amniotic fluid had coefficients of −1.510 and −45.982, respectively, with the regression accounting for 100% of the log likelihood.

Comment

The measurement of lung length seems to be useful in the management of the patient with ruptured membranes, correctly identifying >90% of the patients at risk of pulmonary hypoplasia. With the addition of amniotic fluid volume it was possible to correctly classify 100%. Lung length measurement in our hands was a better predictor of pulmonary hypoplasia than was chest circumference. The measurement is reproducible with high-resolution ultrasonographic equipment and experienced observers. There was a good correlation

between lung length measured by ultrasonography and lung weight postmortem. The normal values can also be applied to situations of diaphragmatic hernia, pleural effusion, and hydrops to accurately measure lung size. There seems to be reduction in lung size after drainage of liquor in some cases but in only one case (patient No. 18, Table I) was the lung length below 2 SDs within 1 week of rupture, and the diagnosis of pulmonary hypoplasia was confirmed postmortem, suggesting the possibility of more prolonged pathology than was thought. These measurements were made on the left lung and should be applied to it. However, we found no significant difference between lungs in length, and if the left lung is difficult to measure then measurements from the right lung can be used.

The next most useful predictor of pulmonary hypoplasia was the amount of amniotic fluid. In all cases with amniotic fluid pockets averaging >1.5 cm the lungs grew normally. If no amniotic fluid was seen there was pulmonary hypoplasia in four of four fetuses (Table I). This contrasts with recent data[13] in a study of 88 infants with ruptured membranes of >7 days in which no correlation was found between the degree of oligohydramnios and pulmonary hypoplasia. In that study, however, the data were retrospective and the ultrasonographic scans performed by different centers with the analysis in some instances being made from ultrasonographic reports. Amounts of amniotic fluid are difficult to assess and considerable experience is required. All scans in our study were performed by the authors. In the study by Rotschild et al.[13] only the last ultrasonographic study was analyzed, whereas we averaged the amount of amniotic fluid from all examinations. Nevertheless Rotschild et al.[13] did note that 12 of 14 infants with pulmonary hypoplasia had moderate or severe oligohydramnios (86%) at the last ultrasonographic examination.

If the membranes ruptured before 22 weeks' gestation all fetuses or neonates died and all had pulmonary hypoplasia.

Breathing movement analysis is more complex. In this study we recorded breathing as positive if more than two breaths were noted together. The studies were performed during an ultrasonographic examination and were not accurately quantified. Therefore care should be taken in making inferences from the observations. We can confirm,[11] however, that pulmonary hypoplasia does develop in some fetuses with frequently observed breathing movements (patient No. 6, Table I). This does not contradict the findings of Blott et al.,[10] inasmuch as they defined breathing as only present if the episode lasted 60 seconds, whereas in our study many of the episodes were of shorter duration. The absence of breathing movements was associated with hypoplasia in five of six cases. Certainly persistent absence of breathing movements over several weeks is a poor prognostic sign. However, the mere presence of breathing movements cannot always be taken as a good prognostic sign. This study did not measure the duration of breathing movements or the degree of diaphragmatic excursion and these may be more useful discriminants.

The interrelationships between these various factors and lung growth are not clear, but if absence of amniotic fluid or breathing leads to pulmonary hypoplasia then it should take time. This group of patients suggests that the membranes must be ruptured for ≥3 weeks before significant growth problems occur. However, there were two exceptions to this rule with pulmonary hypoplasia developing in patient No. 5 after 2 weeks of membrane rupture and in patient No. 18 after only 1 week. Perhaps there was in fact more prolonged membrane rupture that was not detected or oligohydramnios for other reasons. Or possibly even short periods of oligohydramnios can lead to pulmonary hypoplasia at critical periods of development.

The patients with ruptured membranes of >1 month are shown in Fig. 3 and illustrate some of the difficulties in forming hypotheses. Patient No. 14 had definite ruptured membranes with continuous leakage but always had reasonable amniotic fluid on ultrasonographic examination, which probably accounts for the normal lung growth. Patient No. 10 had good amniotic pockets of >1.6 cm for the first 3 weeks and then no amniotic fluid for the last week. Patient No. 6 had amniotic fluid pockets of <1.5 cm for the first 4 weeks but later had pockets of >2 cm; patient No. 3 similarly had less amniotic fluid for the first few weeks and then more. In all instances breathing movements were detected on 50% of the examinations or more. In both patients with pulmonary hypoplasia the membrane rupture occurred before 22 weeks' gestation and this seems to be the most important prognostic indicator.

Grateful thanks to Professor A. Scott of the Mathematics Department, Auckland University, for help with the regression equations and analysis of the data.

REFERENCES

1. Wigglesworth JS, Desai R. Is fetal respiratory function a major determinant of perinatal survival? Lancet 1982;1:264-7.
2. Nimrod C, Varela-Gittings F, Machin G, Campbell D, Wesenberg R. The effect of very prolonged membrane rupture on fetal development. AM J OBSTET GYNECOL 1984;148:540-3.
3. Potter EL. Bilateral renal agenesis. J Pediatr 1946;29:68-73.
4. Reid LM. Lung growth in health and disease. Br J Dis Chest 1984;78:113-8.
5. Roberts AB, Clarkson PM, Pattison NS, Jamieson MG, Mok PM. Fetal hydrothorax in the second trimester of pregnancy: successful intrauterine treatment at 24 weeks gestation. Fetal Therapy 1986;1:203-9.

6. Vintzileos AM, Campbell WA, Rodis JF, Nochimson DJ, Pinette MG, Petrikovsky BM. Comparison of six different ultrasonographic methods for predicting lethal fetal lung hypoplasia. Am J Obstet Gynecol 1989;161:606-12.
7. Nimrod C, Davies D, Iwanicki S, Harder J, Persaud D, Nicholson S. Ultrasound prediction of pulmonary hypoplasia. Obstet Gynecol 1986;68:495-8.
8. Johnson A, Callan N, Bhutani VK, Colmorgen GHC, Weiner S, Bolognese RJ. Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with pulmonary hypoplasia. Am J Obstet Gynecol 1987;157:764-9.
9. DeVore GR, Horenstein J, Platt LD. Fetal echocardiography. VI. Assessment of cardiothoracic disproportion. Am J Obstet Gynecol 1986;155:1066-71.
10. Blott M, Greenough A, Nicolaides Kh, Moscoso G, Gibb D, Campbell S. Fetal breathing movements as predictor of favorable pregnancy outcome after oligohydramnios due to membrane rupture in second trimester. Lancet 1987;2:129-31.
11. Moessinger AC, Higgins A, Fox HE, Rey HR, Haideri M Al. Fetal breathing movements are not a reliable predictor of continued lung development in pregnancies complicated by oligohydramnios. Lancet 1987;2:1297-300.
12. Askenazi SS, Perlman M. Pulmonary hypoplasia: lung weight and radial alveolar count as criteria of diagnosis. Arch Dis Child 1979;54:614-8.
13. Rotschild A, Ling EW, Puterman ML, Farquharson D. Neonatal outcome after prolonged preterm rupture of the membranes. Am J Obstet Gynecol 1990;162:46-52.

Color flow Doppler—A useful instrument in the diagnosis of vasa previa

James A. Harding, MD, David F. Lewis, MD, Carol A. Major, MD, Michael Crade, MD, Jagdish Patel, MD, and Michael P. Nageotte, MD

Long Beach and Orange, California

Vasa previa is associated with an increased perinatal mortality rate and rarely is diagnosed in the antepartum period. We present a case in which vasa previa was correctly diagnosed by use of color flow Doppler imaging. This modality is a valuable adjunct in the evaluation of patients suspected to have vasa previa. (Am J Obstet Gynecol 1990;163:1566-8.)

Key words: Vasa previa, Doppler, color flow Doppler, prenatal diagnosis

Vasa previa is a rare condition in which a placental blood vessel crosses an area of the amnion overlying the cervical os. When this vessel is severed, the fetus can exsanguinate in a matter of minutes; thus the condition is associated with a high fetal mortality rate. The antenatal diagnosis of vasa previa is rarely reported. Ultrasonography and Doppler imaging have been used to diagnose vasa previa in the antenatal period.[1] We report the correct diagnosis of vasa previa by means of color flow Doppler imaging in a patient during the antenatal period.

Case report

A patient (gravida 3, para 1-0-1-1) who was Rh-positive was admitted at a gestational age of 24⅐ weeks with vaginal bleeding. The vaginal bleeding, estimated at 1000 ml, ceased after admission. The patient refused all blood products for religious reasons. Ultrasonography revealed a left posterior marginal placenta previa with a right anterior secondary lobe thought to be succenturiate. Doppler flow evaluation of the region between the placental beds revealed an area of pulsatile flow. Color flow Doppler imaging delineated a vessel coursing across the cervical os with pulsations corresponding to the fetal heart rate (Fig. 1).

Because of the concern over fetal risk of acute blood loss with spontaneous rupture of membranes, a controlled delivery by cesarean section was planned. Amniocentesis was performed at 34 weeks' gestation and fetal pulmonary maturity was established.

At delivery a normal appearing female with a birth weight of 1975 gm and Apgar scores of 8 and 9 at 1 and 5 minutes, respectively, was delivered without interrupting the placental integrity. Initial hematocrit of the baby was 58%. An intact large-bore vessel was identified coursing across the cervical os from the marginal left-posterior previa to the right-anterior succenturiate lobe (Fig. 2).

The postoperative course was uncomplicated, and the patient was discharged home with her infant on the sixth postoperative day.

From the Departments of Maternal-Fetal Medicine and Ultrasound, Memorial Medical Center of Long Beach, and the Department of Maternal-Fetal Medicine, University of California, Irvine, Medical Center.
Received for publication June 19, 1990; accepted June 22, 1990.
Reprint requests: James A. Harding, MD, Department of Maternal-Fetal Medicine, Building 25, University of California, Irvine, Medical Center, 101 The City Dr., Orange, CA 92668.
6/1/23356

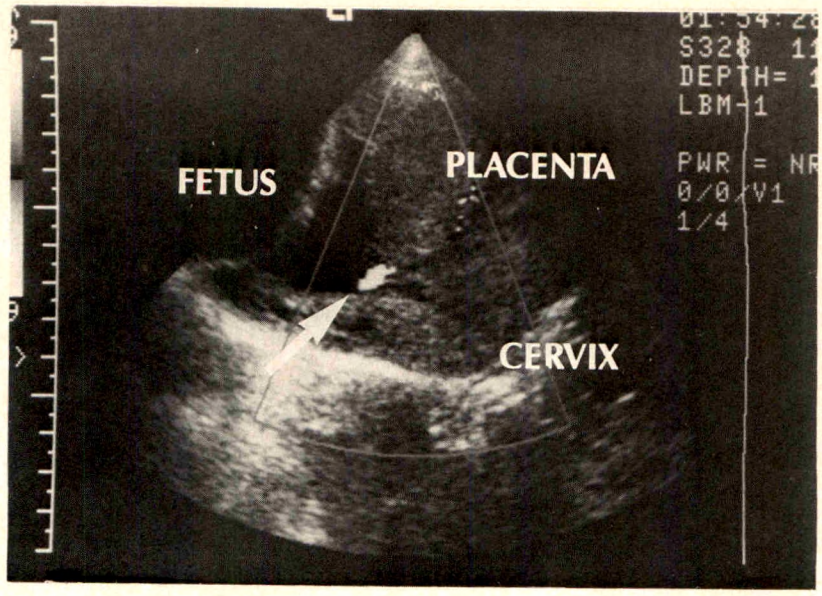


Fig. 1. Ultrasonogram at 25 weeks' gestation. *Arrow* indicates area of flow activity overlying cervical os.

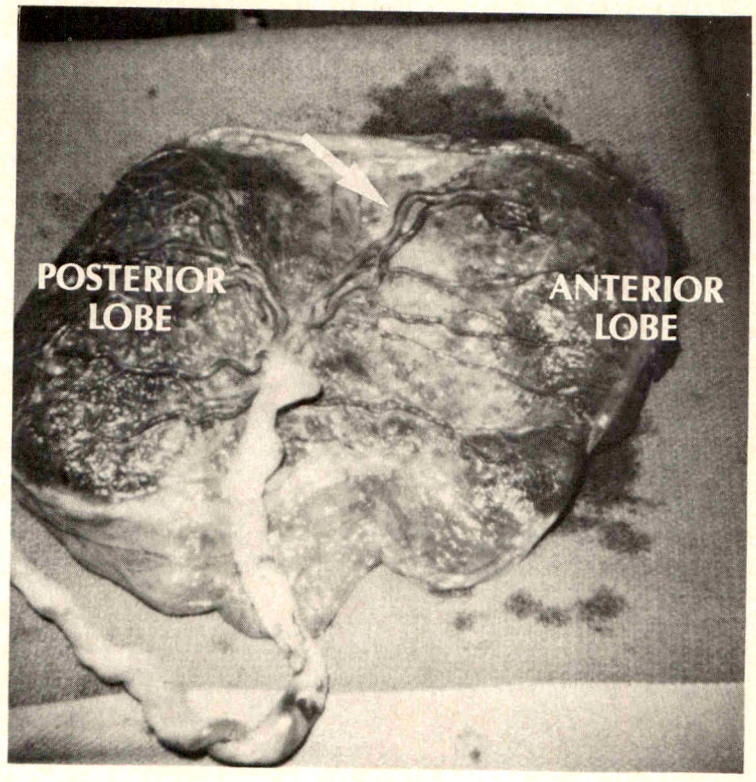


Fig. 2. Postpartum examination of placenta with large vessels coursing across membranes indicated by *arrow*.

Comment

Vasa previa is associated with a high fetal mortality rate. The incidence is approximated to be one in 2000 to 3000 deliveries.[2] Diagnosis of vasa previa has always remained elusive to the clinician. In 1979 Pent[3] outlined the strategies for diagnosing vasa previa at that time. His discussions focused on the diagnosis of vasa previa during the intrapartum period, which involved methods to identify fetal bleeding after the vessel had ruptured. In his report he raised the issue of the possible

use of ultrasonography for the antenatal diagnosis of vasa previa, but thought that the technology at that time could not clearly delineate vessels so deep in the pelvis. In 1987 Gianopoulos et al.[1] reviewed a case report of a succenturiate lobe with a vasa previa detected by ultrasonography and Doppler flow imaging to accurately identify the condition before delivery.

Advancements in Doppler technology include the recent evolution of color flow Doppler. Color flow Doppler imaging use computer-generated colored imprints linked to Doppler signals that correlate with directional vascular flows. The benefits of this tool include identification of smaller vessel flows that initially may escape the scanning M-mode resolutions and standard Doppler techniques.

Maintaining a high index of suspicion is the key to diagnosis of vasa previa. In this respect, the patient with identified ultrasonographic findings at risk for a vasa previa (such as a low-lying to marginal placenta previa, velamentous insertion of the cord, succenturiate lobes of the placenta, or multiple gestations) should warrant color flow Doppler evaluation of the cervical region to rule out a vasa previa. Although the precision of this technique needs further evaluation, it holds promise for improvements in the detection of this highly fatal condition.

REFERENCES

1. Gianopoulos J, Carver T, Tomich P, Karlman R, Gadwood K. Diagnosis of vasa previa with ultrasonography. Obstet Gynecol 1987;69:488-91.
2. Pent D. Vasa previa. Am J Obstet Gynecol 1979;134: 151-5.

Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants

Marlin D. Mills, MD,[a] Michael P. Nageotte, MD,[b] John P. Elliott, MD,[a] Michael Crade, MD,[b] and Wendy Dorchester, MPH[b]

Phoenix, Arizona, and Long Beach, California

Antenatal management of very-low-birth-weight infants often requires difficult obstetric decisions. This study was designed to evaluate the predictive value for neonatal outcome of antenatally acquired estimation of gestational age and ultrasonographically estimated fetal weight or a combination of both in very-low-birth-weight infants. Sixty-seven fetuses with estimated gestational ages between 22⁶/₇ and 28⁶/₇ weeks were studied ultrasonographically to estimate fetal weight. A comparison of accuracy of estimated fetal weight with actual birth weight showed good correlation ($r = 0.93$). Neonatal outcome of these infants was analyzed by estimated gestational age and estimated fetal weight. Estimated gestational age and estimated fetal weight >25 weeks and >750 gm were associated with 50% survival, respectively. However, when both of these conditions were met survival reached 85%. This information may be useful to guide antepartum management decisions in this very-low-birth-weight group. (Am J Obstet Gynecol 1990;163:1568-74.)

Key words: Very-low-birth-weight infant survival, ultrasonographically estimated fetal weight, gestational age versus ultrasonographically estimated fetal weight

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Good Samaritan Medical Center, Phoenix, and the Division of Maternal-Fetal Medicine, Memorial Hospital Medical Center, Long Beach.
Received for publication June 21, 1989; revised June 28, 1990; accepted July 5, 1990.
Reprint requests: Marlin D. Mills, MD, Division of Maternal-Fetal Medicine, Good Samaritan Medical Center, 1111 East McDowell Road, Phoenix, AZ 85006.
6/1/23635

Delivery of a premature infant with a birth weight <1500 gm occurs in approximately 3.9% of all pregnancies. However, this group accounts for 75% of the total perinatal mortality.[1] Over the last several years, significantly better long-term survival statistics of infants born at earlier gestational ages and lower birth weights have been reported.[2,3] In addition to improved neonatal technologies and expertise, there are other

factors beyond the control of the neonatal team that also appear to have significant influence on the outcome of these infants. These include actual birth weight, estimated gestational age, abnormal fetal growth patterns, fetal acidosis, mode of delivery, and location of delivery.[4] The obstetrician's perception of potential viability influences these factors and determines whether aggressive obstetric management, including transfer to a regional perinatal center, or the performance of a cesarean delivery for fetal indications will be used.

Perinatal mortality has been shown to be related to both gestational age and actual birth weight, although each may have an independent effect.[5] Actual birth weight statistics have limited usefulness to the obstetrician, however, because the actual birth weight is unknown before delivery and cannot be used to guide obstetric management. Clinically, fetal weight is often underestimated when compared with actual birth weight. When this occurs, aggressive obstetric intervention, which would otherwise be indicated, is often withheld, resulting in potentially worsened perinatal outcome.[6] Early attempts to antenatally predict actual birth weight by ultrasonographic measurements of various fetal structures and to thereby improve clinical estimated fetal weight were based on studies of infants with actual birth weights >1000 gm.[7,8] Formulas to estimate fetal weight may vary in accuracy at different weight ranges and therefore need to be evaluated for the very-low-birth-weight group.[9]

The usefulness of the antenatal estimation of gestational age is limited because of the frequency with which accurate menstrual dating information is unavailable. Neonatal estimate of gestational age is not accurate enough in the very-low-birth-weight group to allow development of survival tables useful for antenatal management.[10] Ultrasonographic estimation of gestational age also has been shown to have a significantly large range of error and thus limits the sole application of this technique for prospective management of these patients.[11]

The focus of this study was to determine the accuracy of ultrasonographically estimated fetal weight with available formulas to predict actual birth weight in the very-low-birth-weight fetuses. The following question was then asked: could this estimate of fetal weight predict neonatal outcome when compared with or combined with the estimated gestational age?

Material and methods

Women admitted to the labor and delivery suite at Long Beach Women's Hospital from Oct. 1, 1983, through May 30, 1985, with a diagnosis of threatened preterm delivery and a gestational age between 22⅞ weeks and 28⅞ weeks were initially evaluated. Threatened preterm delivery was defined as one of the following: a diagnosis of preterm labor treated with intravenous tocolysis, with or without cervical change; preterm premature rupture of membranes; or a maternal complication necessitating delivery in the above gestational age range. Gestational age was determined by calculating from the patient's last menstrual period, by early physical examination or early examination by ultrasonography, or by ultrasonographic measurements of the fetus taken during the current hospital admission if this was the only information available. Neonatal assessment of gestational age was not used for analysis because of its inaccuracy in this age group.

All patients had an obstetric ultrasonographic examination at the time of admission. The equipment used for this examination was an Acuson real-time ultrasonographic scanner, an ATL-3000-1C real-time ultrasonographic scanner, and a Toshiba SAL-20 portable real-time ultrasonographic scanner. All images were recorded in multiimage format on radiographic transparency film. Fetal ultrasonographic measurements for estimation of gestational age and estimation of fetal weight included biparietal diameter, head circumference, femur length, and abdominal circumference. Biparietal diameter was measured according to the method described by Campbell and Thoms[12] and further defined by Hadlock et al.[13] The occipitofrontal diameter and head circumference were determined from measurements made at this level. Head circumference was determined by the method described by Hadlock et al.[14] using a transverse and longitudinal diameter. The femur length was measured as described by Hadlock et al.[15] Abdominal circumference was calculated by two methods. First, a hand-held map reader was used to trace the abdominal circumference on the radiographic transparency. The second method used transverse diameters as described by Campbell and Thoms.[12] Each method of measuring abdominal circumference was then compared for accuracy. Additional information obtained from the ultrasonographic examination included amniotic fluid volume and fetal presentation. The cephalic index was calculated by the method described by Hadlock et al.[13] with a normal range (±2 SDs) of 70% to 86%. All parameters were analyzed for effect on the accuracy of estimated fetal weight prediction.

Fetal weight was estimated according to two previously published formulas.[7,8] Each formula was calculated with the abdominal circumference measured by the two methods described (Shepard I, Warsof I—map reader; Shepard II, Warsof II—transverse diameters). These estimates were then compared with actual birth weight for prediction accuracy.

The ultrasonographically estimated fetal weight was blinded from the managing obstetrician before delivery. Management decisions were based solely on the

Table I. Comparisons of actual birth weight and calculated values for all infants

	Birth weight (gm, mean ± SD)	*Correlation with actual birth weight (r)*
Actual birth weight	792.7 ± 223.6	1.0000
Shepard EFW I	765.7 ± 219.6	0.9011
Warsof EFW I	807.6 ± 233.0	0.9010
Shepard EFW II	793.6 ± 242.2	0.9309
Warsof EFW II	839.8 ± 258.2	0.9287

EFW, Estimated fetal weight. Comparison among five correlation coefficients shows no statistical significant differences.

estimated gestational age. Cesarean delivery was performed for fetal indication (i.e., malpresentation, or fetal distress) only if the estimated gestational age was considered to be ≥26⅞ weeks. Although cesarean section was not performed for infants with an estimated gestational age <26⅞ weeks, these patients were still managed with care to provide a controlled vaginal delivery. Continuous external fetal monitoring was used. Patients were positioned to reduce the occurrence of variable decelerations as a result of cord compression. Oxygen was provided to the mothers if there was an abnormal fetal heart rate pattern, and hydration was used to improve uterine perfusion.

Actual birth weight was recorded after delivery. Neonatal outcome was evaluated for mortality, length of hospital stay, incidence of respiratory distress syndrome, retrolental fibroplasia, bronchopulmonary dysplasia, sepsis, necrotizing enterocolitis, intraventricular hemorrhage, seizures, and patent ductus arteriosus necessitating surgical ligation. The incidence of these complications was then correlated with estimated gestational age, actual birth weight, and estimated fetal weight. An analysis of neonatal mortality data at the neonatal intensive care unit at Long Beach Women's Hospital, as well as data from this study, showed that survival >50% occurred in the 700 to 800 gm actual-birth-weight group. A birth weight of 750 gm was chosen to represent this group. Outcome was then analyzed for birth weights above and below this weight and compared with ultrasonography-estimated fetal weight, and estimated gestational age for survival and morbidity.

The data were subjected to statistical analysis by means of χ^2 with Fisher's exact test and Yates' correction where appropriate.

Results

Ninety-eight patients were admitted and subsequently delivered during the study period with a gestational age between 22⅞ and 28⅞ weeks. Thirty-one patients were excluded from analysis because of ultrasonographic examination >72 hours from delivery, absent or inadequate ultrasonographic examination, intrauterine growth retardation diagnosed before delivery, congenital anomalies diagnosed before delivery, or multiple gestation. A total of 67 patients were available for analysis. Gestational age was determined by last menstrual period with confirming ultrasonographic examinations in 60% of the patients, early ultrasonographic examination with follow-up examination in 31%, and late ultrasonographic examination only in 9% of patients. There was no difference in outcome among these three groups. The maternal factors responsible for preterm delivery included premature rupture of membranes, premature labor, severe preeclampsia, abruptio placentae, chorioamnionitis, and incompetent cervix. Maternal diagnosis did not influence neonatal outcome when corrected for gestational age and birth weight, although some groups were too small for adequate statistical analysis.

Comparison of the two methods for calculation of abdominal circumference showed no difference by method type. Although the formula that correlated best with actual birth weight was the Shepard II formula, there was no statistically significant difference among each of the formulas evaluated (Table I). The Shepard II formula was used for subsequent comparisons with estimated gestational age and actual birth weight for analysis of outcome. Comparison of formulas for the prediction of infants weighing >750 gm and <750 gm showed no significant difference between the two groups (Table II). In the pregnancies with estimated fetal weights >750 gm, the Shepard formula I had the best correlation with actual birth weight. There was significant correlation of actual birth weight with ultrasonographic estimate of fetal weight for each gestational age group (Table III). Gestational age, actual birth weight, interval between ultrasonographic examination and delivery, fetal presentation, amniotic fluid volume, cephalic index, and fetal sex were analyzed for their potential effect on the accuracy of estimated fetal weight in predicting actual birth weight and were found to have no statistically significant influence.

Evaluation of survival by weight groups shows a significant increase in survival between the 700 and 800 gm and the 800 and 900 gm weight groups (Table IV). Therefore the 700 to 800 gm weight group was further divided into groups of ≤750 gm or >750 gm. Survival was better in the >750 gm weight group (Table V).

Evaluation of survival by gestational age showed the 50% survival breakpoint to occur at 25 weeks' gestation (Table VI). Survival was analyzed for both gestational age of <25 weeks or ≥25 weeks and estimated fetal weight of ≤750 gm or >750 gm; results are shown in Table VII. Survival using estimated gestational age ≥25

Table II. Comparisons of actual birth weight and calculated values for infants with birth weighs >750 gm and ≤750 gm

	Actual birth weight ≤750 gm		*Actual birth weight > 750 gm*	
	Birth weight (gm, mean ± SD)	*Correlation with actual birth weight (R)*	*Birth weight (gm, mean ± SD)*	*Correlation with actual birth weight (R)*
Actual birth weight	590.8 ± 114.2	1.0000	946.7 ± 151.7	1.0000
Shepard EFW I	622.9 ± 126.1	0.7865	1005.4 ± 204.5	0.8557
Warsof EFW I	590.4 ± 115.8	0.7952	948.7 ± 193.3	0.7418
Shepard EFW II	616.5 ± 114.6	0.8098	953.4 ± 190.9	0.7738
Warsof EFW II	585.6 ± 107.2	0.8056	903.2 ± 180.2	0.7759

EFW, Estimated fetal weight. Comparison among five correlation coefficients shows no statistically significant differences.

Table III. Weight comparison by estimated gestational age

Weeks' gestation	*n*	*Actual birth weight (gm, mean ± SD)*	*Estimated fetal weight (gm, mean ± SD)*	*r*	*p*
<24	13	518 ± 78.37	566 ± 126.15	0.746	0.003
24	7	721.4 ± 101.9	689.4 ± 72.86	0.844	0.017
25	16	701 ± 122	723.1 ± 137.2	0.903	0.001
≥26	31	971.2 ± 163.5	946.6 ± 242.1	0.769	0.001
TOTAL	67	792 ± 223.6	808 ± 233.5	0.901	0.001

weeks alone was 70%. Survival using estimated fetal weight >750 gm alone was 80.5%. No difference was found in the occurrence of neonatal complications among the surviving infants when compared by actual birth weight, estimated fetal weight, or estimated gestational age, although this may be the result of the small number of cases in each group.

Comment

Several studies have documented the improved survival and long-term outcome of very-low-birth-weight infants.[2, 3] These studies have attempted to correlate neonatal mortality risk in relation to birth weight and gestational age groups, on the basis of actual birth weight and neonatal assessment of gestational age, neither of which is available to the obstetrician who manages the patient before delivery.[16] Paul et al.[6] demonstrated that there was a significant increase in neonatal mortality when the actual birth weight of the infant was underestimated before delivery. Their conclusion was that obstetricians must be aware of the tendency to underestimate fetal size on the basis of the clinical examination and that outcome might be improved by intervening for fetuses estimated to weigh ≥750 gm. Goldenberg et al.[17] reported the results of a survey of all physicians in Alabama performing obstetric care in 1978. These practicing obstetricians estimated that the 50% and the 90% survival rates were reached nearly 2 weeks later than was actually true at both their individual hospitals and the regional perinatal centers. This study emphasized the need for all physicians who practice obstetrics not only to be aware of the morbidity and mortality statistics in their hospital and region but also to be willing to manage these patients aggressively at these early gestational ages. Studies reviewing mortality rates in the very-low-birth-weight group show that with aggressive obstetric management the 50% neonatal survival has changed to an estimated gestational age and birth weight of 26 weeks and 750 gm.[1, 18] These studies also emphasize the need for aggressive management at lower gestational ages and suggest that under special circumstances aggressive management may be justified even for those infants judged to have an estimated gestational age <26 weeks.

In an attempt to study individual factors that influence survival and morbidity with very-low-birth-weight deliveries, Worthington et al.[19] presented data using actual birth weight that showed improved survival and prognosis of infants with birth weights of ≥750 gm or gestational ages >25 weeks. Their data showed birth weight to be significantly correlated with survival independent of gestational age.

At the time of our study there were no available data regarding the accuracy of ultrasonographic predictions of estimated fetal weight or a correlation of these predictions with neonatal outcome in our institution. We needed to determine the best available ultrasonographic formula for predicting actual birth weight

Table IV. Survival by weight groups

Weight (gm)	*Actual birth weight*			*Estimated fetal weight*		
		Survivors			*Survivors*	
	n	*No.*	*%*	*n*	*No.*	*%*
≤600	13	0	0	13	0	0
601-700	10	2	20	10	4	40
701-800	16	7	44	15	6	40
801-900	7	7	100	7	6	86
901-1000	6	5	83	9	7	78
≥1001	14	14	100	13	13	100
TOTAL	67	35	52	67	35	52

Table V. Survival in 701 to 800 gm weight range by 50 gm interval

Weight	*Actual birth weight*			*Estimated fetal weight*		
		Survivors			*Survivors*	
	n	*No.*	*%*	*n*	*No.*	*%*
≤750 gm	5	1	20	8	2	25
>750 gm	11	6	54.5	7	4	57.1
TOTAL	16	7	43.8	15	6	40

Table VI. Survival comparison by estimated gestational age

Gestation (wk)	*n*	*Survivors*	
		No.	*%*
≤24	13	0	0
24	7	2	28.6
25	16	8	50.0
≥26	31	25	80.6
TOTAL	67	35	52.0

given the clinical circumstances in our institution. At that time formulas by Shepard et al.[7] and Warsof et al.[8] had been published. In our population the formula by Shepard et al., using two transverse diameters for calculation of the abdominal circumference (Shepard II), had the greatest correlation with actual birth weight, although there was no statistically significant difference among the four formulas. Key et al.[20] used similar formulas and found a high degree of correlation between ultrasonography-estimated fetal weight and actual birth weight. Their data also did not reach statistically significant difference, but they found the formula published by Warsof et al. to have a slightly greater correlation in their population. The difference between these formulas is important; we found the Warsof formula used by Key et al. to consistently estimate a lower fetal weight when compared with the formula published by Shepard et al. Using the formula of Shepard et al. instead of the formula by Warsof et al. may result in increased aggressive intervention. Since the initiation of this study other formulas have been published in the very-low-birth-weight group in an attempt to improve the accuracy of estimated fetal weight. Pielet et al.[21] showed that of the formulas evaluated that of Shepard et al. used in our study was one of four with better correlation, but the three formulas with slightly greater correlation included measurements not only of biparietal diameter and abdominal circumference but of femur length as well. It is important to use all available measurement parameters for the calculation of estimated fetal weight when available to attempt to achieve the greatest correlation.

As in the report by Key et al.,[20] our data did not show any significant effect on the accuracy of estimated fetal weight prediction caused by fetal malpresentation, amniotic fluid volume, or cephalic index.

In comparison of actual birth weight, estimated fetal weight, and estimated gestational age with survival, our data show that there was no difference in outcome when estimated fetal weight and actual birth weight were compared.

Our data also showed that when estimated gestational age and estimated fetal weight alone and in combination were compared, there was survival of ≥50% at a gestational age of 25 weeks (Table VI); when estimated fetal weight was evaluated, survival of ≥50% occurred at >750 gm (Table V). The effect of gestational age was independent of that of estimated fetal weight

Table VII. Survival by estimated gestational age and estimated fetal weight

Estimated gestational age/estimated fetal weight	*n*	*Survivors*	
		No.	*%*
<25 wk/≤ 750 gm	18	2	11.1
<25 wk/>750 gm	2	0	0
≤25 wk/≤750 gm	13	4	30.8
≥25 wk/>750 gm*	34	29	85.3

*Significantly different from other groups, $p = 0.001$.

(Table VII); however, if the estimated fetal weight was ≤750 gm, gestational age did not affect survival. When the estimated fetal weight was >750 gm, a gestational age ≥25 weeks was associated with improved survival. If the gestational age was <25 weeks, survival was not affected by the estimated fetal weight. However, if the gestational age was ≥25 weeks, an estimated fetal weight of >750 gm was associated with increased survival to a significant degree ($p = 0.001$). Patients in the groups associated with decreased potential survival must be managed individually. Careful attention to the management of labor, including position changes, hydration, analgesia, and oxygenation may improve neonatal outcome of these infants, particularly if there has been an underestimation of the potential for survival. Chervenak et al.[22] also reported that gestational age is more important than neonatal weight in predicting a good outcome. Howver, in the prediction of adverse neonatal outcome, both the estimated fetal weight and obstetric gestational age were significant predictors.

In conclusion, our data support a significant correlation between the ultrasound estimate of fetal weight and actual birth weight. There is no significant difference in survival and morbidity in comparison of actual birth weight with estimated fetal weight as calculated by the formula by Shepard et al. using transverse diameters to calculate abdominal circumference. In our institution, estimated gestational age and estimated fetal weight were associated independently with a 50% neonatal survival at ≥25 weeks and >750 gm, respectively. More important, if the infant had an antenatal estimated gestational age of ≥25 weeks and an ultrasonographically predicted estimated fetal weight >750 gm, neonatal survival reached 85%.

This information can be used to help guide the obstetrician in management decisions regarding the care of these very-low-birth-weight infants. In patients with uncertain dates, the ultrasonography-estimated fetal weight alone may be a better parameter to guide antepartum management. Pregnancies that are <25 weeks or <750 gm estimated fetal weight must be managed on the basis of survival data that are <50%, although careful labor management may improve outcome in this group.

Continued study is needed in this area to better clarify the potential limits of viability on the basis of both estimated gestational age and predicted fetal weight. Formulas that provide the greatest degree of correlation for the various birth weights still need to be evaluated and modified for a closer fit whenever possible. We are currently evaluating a population of patients who have been managed aggressively according to the results of this study and supported by recently published studies that also show improved outcome of infants in the same age and weight ranges.

We thank Tari Radin, RN, MSN, PhD, for assistance with the statistical analysis.

REFERENCES

1. Koops BL, Morgan LJ, Battaglia FC. Neonatal mortality risk in relation to birth weight and gestational age: update. J Pediatr 1982;101:969-77.
2. Hack M, Fanaroff A. How small is too small? Considerations in evaluating the outcome of the tiny infant. Clin Perinatol 1988;15:773-89.
3. Luthy DA, Shy KK, Strickland D, et al. Status of infants at birth and risk for adverse neonatal events and long term sequelae: a study in low birth weight infants. Am J Obstet Gynecol 1987;157:676-9.
4. Amon E, Sibai BM, Anderson GD, et al. Obstetric variables predicting survival of the immature newborn (<1000 gm): a five-year experience of a single perinatal center. Am J Obstet Gynecol 1987;156:1380-9.
5. Williams RL, Creasy RK, Cunningham GC. Fetal growth and perinatal viability in California. Obstet Gynecol 1982;59:624-32.
6. Paul RH, Koh KS, Monfared AH. Obstetric factors influencing outcome in infants weighing from 1,001 to 1,500 grams. Am J Obstet Gynecol 1979;133:503-8.
7. Shepard MJ, Richards VA, Berkowitz RL, et al. An evaluation of two equations for predicting fetal weight by ultrasound. Am J Obstet Gynecol 1982;142:47-54.
8. Warsof SL, Gohari P, Berkowitz RL, et al. The estimation of fetal weight by computer-assisted analysis. Am J Obstet Gynecol 1977;128:881-92.
9. Eden RD, Jelovsek FR, Kodack LD, et al. Accuracy of ultrasonic fetal weight prediction in preterm infants. Am J Obstet Gynecol 1983;147:43-7.
10. Spinnato JA, Sibai BM, Shaver DC, et al. Inaccuracy of Dubowitz gestational age in low birth weight infants. Obstet Gynecol 1984;63:491-5.
11. Sabbagha RE, Hughey N. Standardization of sonor cephalometry and gestational age. Obstet Gynecol 1978; 52:402-6.
12. Campbell S, Thoms A. Ultrasound measurement of the fetal head to abdominal circumference ratio in the as-

sessment of growth retardation. Br J Obstet Gynaecol 1977;84:165-74.
13. Hadlock FP, Deter RL, Harrist RB, et al. Fetal biparietal diameter: rational choice of plane of section for sonographic measurement. Am J Radiol 1982;138:871-4.
14. Hadlock FP, Kent WR, Lloyd J, et al. An evaluation of the methods for measuring fetal head and body circumferences. J Ultrasound Med 1982;1:359-64.
15. Hadlock FP, Harrist RB, Deter RL, et al. Fetal femur length as a predictor of menstrual age: sonographically measured. Am J Radiol 1982;138:875-8.
16. Goldenberg RL, Nelson KG, Hale CD, et al. Survival of infants with low birth weight and early gestational age, 1979 to 1981. AM J OBSTET GYNECOL 1984;149:508.
17. Goldenberg RL, Nelson KG, Dyer RL, et al. Variability of viability: the effect of physicians' perception of viability on the survival of very low birth weight infants. AM J OBSTET GYNECOL 1982;143:678-84.
18. Herschel M, Kennedy JL, Kayne HL, et al. Survival of infants born at 24 to 28 weeks' gestation. Obstet Gynecol 1982;60:154-8.
19. Worthington D, Davis LE, Gravez JP, et al. Factors influencing survival and morbidity with very low birth weight delivery. Obstet Gynecol 1983;62:550-5.
20. Key TC, Dattel BJ, Resnick R. The ultrasonographic estimation of fetal weight in the very low birth weight infant. AM J OBSTET GYNECOL 1983;145:574-8.
21. Pielet BN, Sabbagha RE, MacGregor SN, et al. Ultrasonic prediction of birth weight in preterm fetuses: which formula is best? AM J OBSTET GYNECOL 1987;157:1411-4.
22. Chervenak FA, Berkowitz GS, Thornton J, et al. A comparison of sonographic estimation of fetal weight and obstetrically determined gestational age in the prediction of neonatal outcome for the very low birth weight fetus. AM J OBSTET GYNECOL 1985;152:47-50.

Management of selected cystic adnexal masses in postmenopausal women by operative laparoscopy: A pilot study

William H. Parker, MD, and Jonathan S. Berek, MD
Los Angeles, California

Twenty-five postmenopausal patients were predicted to have benign masses by screening criteria that included ultrasonographic findings of a cystic adnexal mass <10 cm with distinct borders and no evidence of irregular solid parts, thick septa, ascites, or matted bowel, and a normal serum CA 125 value (<35 U/ml). All 25 masses were accurately predicted to be benign. The size of the cysts on ultrasonography ranged from 3 to 9 cm with a mean of 5 cm. Twenty-two patients (88%) were successfully managed by operative laparoscopy and adnexectomy. Mean operative time was 70 minutes and mean postoperative hospital stay was 12 hours. Mean time of return to normal activity was 5 days. Three patients required laparotomy. We conclude that removal of these cystic adnexal masses by operative laparoscopy is an acceptable alternative in carefully selected postmenopausal women. (AM J OBSTET GYNECOL 1990;163:1574-7.)

Key words: Postmenopausal, adnexal mass, operative laparoscopy

The standard operative approach for adnexal masses found in postmenopausal women has been the performance of an exploratory laparotomy that allows early detection, resection, and proper staging if ovarian cancer is found. Total abdominal hysterectomy and contralateral salpingo-oophorectomy also have been advocated in the presence of benign adnexal neoplasms because of the concern for the subsequent development of a neoplasm in the contralateral ovary or uterus.[1] However, most adnexal masses in postmenopausal women are benign.[2-4] In addition, the risk of having a subsequent malignancy in a remaining pelvic organ is very low.[5,6]

If a screening process could accurately select masses that were likely to be benign, evaluation and removal with operative laparoscopic techniques would allow outpatient management with less cost and an easier recovery. The safety and efficacy of this approach have been well documented in premenopausal women with adnexal pathology.[7,8] With the use of specific ultrasonographic criteria, several authors[9-11] have been able to

From the Department of Obstetrics and Gynecology, University of California Los Angeles School of Medicine.
Supported by a grant from the Ramona Moskovitz Cancer Fund.
Received for publication April 5, 1990; accepted July 27, 1990.
Reprint requests: William H. Parker, MD, 1450 10th St., Santa Monica, CA 90401.
6/1/24112

accurately predict benign masses in 95% to 100% of postmenopausal patients.

Other authors[12-15] have used the CA 125 assay, which uses a monoclonal antibody that reacts to an antigen found in most nonmucinous ovarian cancers, to predict benign versus malignant pelvic masses. Finkler[16] combined ultrasonographic appearance, CA 125 values, and clinical impression to accurately predict benign masses in all postmenopausal women studied.

With a combination of preoperative ultrasonographic appearance that suggested a benign mass and normal serum CA 125 values, we selected a group of postmenopausal women who had a minimal chance of having an ovarian malignancy. The masses were then approached surgically by operative laparoscopy.

Material and methods

Candidates for this study included postmenopausal women who were found to have an adnexal mass on pelvic examination or noninvasive pelvic imaging studies and who were referred for evaluation and treatment. Postmenopausal status was defined as age over 45 years with at least 12 months of amenorrhea or a follicle-stimulating hormone >40 mIU/ml.

Twenty-five patients were consented for surgery. Patient ages ranged from 47 to 81 years with a mean of 63 years. Time from the last menstrual period ranged from 12 months to 33 years with a mean of 14 years. Eight patients (32%) had undergone prior hysterectomies for benign disease. Ultrasonographic criteria used were those modified from Herrmann.[9] Criteria for the presumptive diagnosis of a benign mass were: (1) size <10 cm; (2) cystic; (3) distinct borders; (4) no irregular solid parts or thick septa; (5) no evidence of ascites, and (6) no matted bowel. The presence of thin septa did not preclude inclusion in this study. Ultrasonographic examination was performed transabdominally with a full bladder and within 4 weeks of operation. Before surgery, one of the authors (W. P.) reviewed all scans with a radiologist for the above criteria. The size of the cysts on ultrasonography ranged from 3 to 9 cm with a mean of 5 cm.

Serum CA 125 values were considered normal if <35 U/ml. All assays were done <2 weeks before surgery and they were done in one laboratory with a radioimmunoassay kit (Centocor, Malvern, Pa.). The CA 125 values ranged from 3 to 33 U/ml with a mean of 18 U/ml.

The patient was required to be an appropriate surgical candidate. Patients were consented for adnexectomy by operative laparoscopy and for possible laparotomy. After general anesthesia was induced they had laparoscopic assessment of the pelvis and abdomen. If there was no evidence of tumor on the ovary or peritoneal surfaces and no evidence of ascites, operative laparoscopy was performed. A 10 mm laparoscope was placed through an infraumbilical incision and two or three accessory instruments were placed through suprapubic incisions. If necessary, lysis of adhesions was performed to free the adnexa. The cystic mass was then aspirated carefully to minimize the potential for fluid spill. As described by Semm[17] three no. 1 catgut endoloops were secured on the infundibulopelvic ligament and the adnexa was resected. The specimen was divided with 5 mm scissors and removed through an 11 mm accessory cannula. Frozen section was done on the removed adnexa. The procedure was terminated when the tissue was benign. If a malignancy were found, we would proceed with immediate staging laparotomy. Laparotomy was also to be performed if necessary to manage a complication or if removal of the mass by operative laparoscopy was not feasible technically.

Results

The operative findings were benign in all cases confirming the preoperative assessment. The histologic assessment of the 25 lesions were as follows: eight serous cysts, six serous cystadenomas, four serous cystadenofibromas, three paratubal serous cysts, three hydrosalpinges, and one benign retroperitoneal serous cyst.

The operative time for those patients managed by operative laparoscopy ranged from 20 to 180 minutes with a mean of 73 minutes. The duration of the postoperative hospital stay for these patients ranged from 3 to 24 hours with a mean of 12 hours. Return to normal activity ranged from 1 to 10 days with a mean of 5 days. One patient underwent operative laparoscopy during the course of a prolonged hospitalization (29 days) for a gastrointestinal and neurologic evaluation, and she is excluded from this analysis.

Two patients had complications of surgery. One patient had a perforation of the bladder with a 5 mm trocar that was managed successfully with at-home catheter drainage for 5 days. Another patient had an injury to the sigmoid colon during lysis of dense adhesions that led to laparotomy for management. Another patient had a frozen section that was inconclusive, thus requiring a laparotomy to complete the removal of a mass that was densely adherent to the pelvic peritoneum. Permanent sections revealed a benign serous cystadenofibroma.

A third patient required laparotomy when laparoscopic assessment revealed a benign paratubal cyst. Incidentally, small papillary excrescences were noted on the contralateral ovary and omentum. Biopsy specimens of these revealed metastatic carcinoma consistent with primary breast cancer that had been diagnosed 5 years earlier. Therefore exploratory laparotomy, bilateral salpingo-oophorectomy, and omentectomy were performed.

Comment

Adnexal masses may be discovered in postmenopausal women during routine examination or the evaluation of gastrointestinal or pelvic complaints. The increased use of pelvic ultrasonography and computed tomography appears to be detecting both symptomatic and incidental masses more frequently.[5] In addition, transvaginal ultrasonography is under study as a screening method for ovarian cancer and has been shown to detect asymptomatic masses that then require investigation.[18]

In 1971 Barber and Graber[19] described the "postmenopausal palpable ovary syndrome" on the basis of three patients found to have ovarian cancer in ovaries that were of normal premenopausal size. They recommended total abdominal hysterectomy, bilateral salpingo-oophorectomy because of the presumed high risk of ovarian cancer in this age group. However, subsequent studies have shown that most of these masses are benign. Flynt and Gallup[20] found that 10 of 11 patients with postmenopausal palpable ovaries had benign processes and one patient had an adenocarcinoma of the bowel. With ultrasonographic criteria similar to those applied in our study, Herrmann[9] was able to accurately predict benign masses in 177 of 185 patients (96%). In addition, all 48 purely cystic tumors <10 cm were benign and 8 of 10 stage I ovarian cancers were accurately predicted. Likewise, Goldstein[11] found that in postmenopausal women, all 28 unilocular cysts <5 cm were benign. Rulin[10] found that in postmenopausal patients, 31 of 32 adnexal masses (97%) <5 cm on ultrasonography were benign, regardless of consistency; whereas masses 5 to 10 cm were benign in 49 of 55 patients (89%). Hall and McCarthy[21] found one borderline neoplasm and 12 benign processes in 13 postmenopausal women with simple adnexal cysts <10 cm. Granberg[4] compared the gross pathologic appearance of ovarian tumors with their histologic diagnosis, and in 116 women over 50 years of age with unilocular cysts, only one (0.08%) was malignant.

CA 125, a tumor-associated surface antigen, has been studied to determine its value in preoperative differentiation of benign and malignant pelvic masses. Vasilev[12] found that 128 of 132 patients (97%) with pelvic masses who had serum CA 125 values <35 U/ml had benign masses. Of the other four patients, two had borderline lesions and only two had cystadenocarcinomas, both of the mucinous type. Einhorn[13] found that 14 of 18 patients with CA 125 values <35 U/ml had benign masses, two had borderline lesions, and two had mucinous cystadenocarcinomas. O'Connell et al.[14] found in a population referred to a tertiary center because of a clinical suspicion of ovarian cancer, all 30 patients who had a CA 125 values <35 U/ml had benign masses. Finkler[16] combined the use of ultrasonography, CA 125 values and clinical impression to evaluate adnexal masses preoperatively and found that all 10 postmenopausal patients predicted to have benign processes were accurately predicted. Similarly, Jacobs[22] found that in nine postmenopausal women with increased ovarian volumes on ultrasonographic examination and CA 125 values <30 U/ml, seven patients had benign cysts and two had a normal pelvis.

Our study also suggests that use of a combined approach with CA 125 values <35 U/ml and an ultrasonogram showing a "benign appearing" mass is a reasonable selection process. In all 25 patients the masses were accurately predicted to be benign.

Some authors have recommended removal of all pelvic organs at the time of exploratory laparotomy in postmenopausal women to remove potentially neoplastic tissue.[2] However, the risk of cancer developing in a remaining ovary that appears normal at laparoscopy in postmenopausal women should be less than the 3 to 5 per 10,000 incidence of ovarian cancer in this age-group.[5] The incidence of uterine cancer is also low in asymptomatic screened postmenopausal women (i.e., approximately 1.7 per 1000).[6] Thus the performance of a laparotomy may be discretionary in selected patients. A contralateral adnexectomy could be performed selectively, although we chose not to remove normal appearing adnexa in these patients because of the added operating time and potential morbidity.

Thought might also be given to adnexectomy by minilaparotomy in this group of patients. However, we believe that evaluation of the entire pelvis and abdomen by the laparoscope is superior to that possible during minilaparotomy. In addition, time for return to full activity should be shorter with operative laparoscopy.

Laparoscopic drainage or removal of these masses in this population has generally been avoided because of the theoretic risk of "spilling" cancer cells into the peritoneal cavity and the fear of decreasing the patients survival,[23] although this has not been documented. Dembo and colleagues[24] have presented recent evidence in a large study of 519 patients with stage I epithelial ovarian cancer that the only factors that influenced the ultimate rate of relapse and survival were the tumor grade, the presence of dense adhesions, and the presence of large volume ascites. When these factors were accounted for in a logistic regression, multivariate analysis the rate of relapse and prognosis was not influenced by rupture of the tumor. Thus the theoretic potential for tumor dissemination by rupture of a malignant cyst remains conjectural.

Some authors[25, 26] have advocated simple drainage of ovarian lesions with the laparoscope or transvaginally with ultrasonographic guidance. Kjellgren[27] found that 10% of aspiration cytologies were read as benign when, in fact, carcinoma was present. Tropé[28] was able to iden-

tify only four of 12 malignant cysts by aspiration cytology, indicating the inadequacy of this procedure for definitive diagnosis. In addition, many of the lesions in our study had functional epithelium and therefore might be expected to persist unless removed.

Goldstein[11] suggested that purely cystic masses <5 cm might be followed up with serial ultrasonographic examinations in seriously ill patients. However, without removal the diagnoses remains in question. By removing the entire adnexa with operative laparoscopy, a complete specimen is provided for precise pathologic analysis and persistence is precluded. Orlando[29] showed that in 345 patients over the age of 60 years who had laparoscopy the complication rate was no different than in 1500 younger patients. Our study also showed that operative laparoscopy is well tolerated in postmenopausal women and that the hospital stay and the time to recovery are shorter than expected from laparotomy. We believe that removal of these adnexal masses is appropriate, and that management by operative laparoscopy allows an acceptable alternative in carefully selected postmenopausal women. More accurate tumor markers and the use of vaginal ultrasonography might enhance the selection process.[30]

REFERENCES

1. Creasman W, Soper J. The undiagnosed adnexal mass after the menopause. Clin Obstet Gynecol 1986;29:446-52.
2. Eriksson L, Kjellgren O, Von Scholtz R. Functional cyst or ovarian cancer: histopathological findings during one year of surgery. Gynecol Obstet Invest 1985;19:155-9.
3. Granberg S, Wikland M, Jannson I. Macroscopic characterization of ovarian tumors and the relation to the histological diagnosis: criteria to be used for ultrasound evaluation. Gynecol Oncol 1989;35:139-44.
4. Berek J, Hacker N. Practical gynecologic oncology. Baltimore: Williams and Wilkins, 1989:328.
5. DiSaia F, Creasman W. Clinical gynecologic oncology. St. Louis: CV Mosby, 1989:311.
6. Koss L, Schreiber K, Oberland S, et al. Detection of endometrial carcinoma and hyperplasia in asymptomatic women. Obstet Gynecol 1964;64:1.
7. Levine R. Economic impact of pelviscopic surgery. J Reprod Med 1985;30:655-9.
8. Nezhat C, Winter W, Nezat F. Laparoscopic removal of dermoid cysts. Obstet Gynecol 1989;73:278-80.
9. Herrman U, Locher G, Goldhirsch A. Sonographic patterns of ovarian tumors: prediction of malignancy. Obstet Gynecol 1987;69:777-81.
10. Rulin M, Preston A. Adnexal masses in postmenopausal women. Obstet Gynecol 1987;70:578-81.
11. Goldsetin S, Subramanyam B, Snyder J, Beller U, Raghavendra N, Beckman E. The postmenopausal cystic adnexal mass: the potential role of ultrasound in conservative management. Obstet Gynecol 1989;73:8-10.
12. Vasilev S, Schlaerth J, Campeau J, Morrow P. Serum CA 125 levels in preoperative evaluation of pelvic masses. Obstet Gynecol 1988;71:751-6.
13. Einhorn N, Bast R, Knapp R, Tjernberg B, Zurawski R. Preoperative evaluation of serum CA 125 levels in patients with primary epithelial ovarian cancer. Obstet Gynecol 1986;67:414-6.
14. O'Connell G, Ryan E, Murphy J, Prefontaine M. Predictive value of CA 125 for ovarian carcinoma in patients presenting with pelvic masses. Obstet Gynecol 1987;70:930-2.
15. Di-Xia C, Schwartz P, Xinguo L, Zhan Y. Evaluation of CA 125 levels in differentiating malignant from benign tumors in patients with pelvic masses. Obstet Gynecol 1988;72:23-7.
16. Finkler N, Benacerrat B, Lavin F, Wojciechowski C, Knapp R. Comparison of serum CA 125, clinical impression, and ultrasound in the preoperative evaluation of ovarian masses. Obstet Gynecol 1988;72:659-664.
17. Semm K. Operative manual for endoscopic abdominal surgery. Chicago: Year Book Medical Publishers, 1987:181-3.
18. Higgins R, van Nagel J, Donaldson E, et al. Transvaginal sonography as a screening method for ovarian cancer. Gynecol Oncol 1989;34:402-5.
19. Barber H, Graber E. The postmenopausal palpable ovary syndrome. Obstet Gynecol 1971;38:921-3.
20. Flynt J, Gallup D. The postmenopausal ovary syndrome: a fourteen-year review. Military Med 1981;146:686-8.
21. Hall D, McCarthy K. The significance of the postmenopausal simple adnexal cyst. J Ultrasound Med 1986;5:503-5.
22. Jacobs I, Stabile I, Bridges J, et al. Multimodal approach to screening for ovarian cancer. Lancet 1988;1:268-71.
23. Purola A, Neeminen V. Does rupture of cystic carcinoma during operation influence the prognosis? Ann Chir Gynaecol 1968;57:615-7.
24. Dembo A, Davy M, Stenwig A, Berle E, Bush R, Kjorstad K. Prognostic factors in patients with stage I epithelial ovarian cancer. Obstet Gynecol 1990;75:263-72.
25. Kleppinger R. Ovarian cyst fenestration via laparoscopy. J Reprod Med 1978;21:16-9.
26. Montanari L, Saviotti C, Zara C. Aspiration of ovarian cysts: laparoscopy or echography? Acta Eur Fertil 1987;18:45-7.
27. Kjellgren O, Angstrom T, Bergman F, Wilkund D. Fine needle aspiration biopsy in diagnosis and classification of ovarian carcinoma. Cancer 1971;28:967-76.
28. Tropé C. The preoperative diagnosis of malignancy of ovarian cysts. Neoplasia 1981;28:117-21.
29. Orlando R, Lirussi F, Nassuato G, Okolicsany L. Complications of laparoscopy in the elderly: a report on 345 consecutive cases and comparison with a younger population. Endoscopy 1987;19:145-6.
30. Leibman A, Kruse B, McSweeney M. Transvaginal sonography: comparison with transabdominal sonography in the diagnosis of pelvic masses. Am J Roentgen 1988;151:89-92.

Hyperadrenergic orthostatic intolerance as a result of postpartum blood loss

Scott L. Mader, MD, Judith A. Mackall, MD, and Martin P. Emert, MD
Cleveland, Ohio

This article describes a young women in whom orthostatic intolerance developed as a result of protracted postpartum vaginal bleeding. The cardiovascular abnormalities were unresponsive to usual treatment measures, and she required combination therapy directed at increasing plasma volume and decreasing sympathetic nervous system activity. After resolution of her symptoms, the patient required no long-term therapy. Chronic blood loss has not been reported as a cause of this syndrome. (Am J Obstet Gyneco. 1990;163:1578-9.)

Key words: Hypotension, postpartum, hyperadrenergic

Orthostatic hypotension usually results from two major causes. It can occur as a result of an inadequate nervous system response that is a result of central or peripheral nervous system dysfunction from disease or medications. Orthostatic hypotension can also occur as a result of excessive stress to the cardiovascular system, such as a decrease in blood volume, or vasodilation. Patients in this latter group often demonstrate a supranormal heart rate response and narrowing of the pulse pressure; the condition is also known as hyperadrenergic orthostatic intolerance. We report a case of hyperadrenergic orthostatic intolerance that developed after chronic postpartum blood loss that was not reversible with volume replacement. Chronic blood loss has not been previously reported as a cause of this disorder.

Case report

The patient was a 30-year-old black woman with no previous medical problems. Six weeks before admission she had an uncomplicated vaginal delivery of her fifth child. Four weeks before admission heavy vaginal bleeding developed and the patient was seen in the emergency room for syncope. She was markedly orthostatic and had a hematocrit value of 17%. The patient had transfusion on two occasions and was admitted 2 weeks later because of continued bleeding. She had curettage that showed retained placental products. She was discharged with iron sulfate therapy, 325 mg three times a day, as her only medication. The patient was seen in the emergency room two additional times within the following week. Both times she was markedly orthostatic and tachycardic with a stable hematocrit level. She was treated with normal saline solution fluid replacement and her symptoms and blood pressure improved. However, her symptoms recurred after several hours.

On the day of admission the patient again complained of dizziness, palpitations, and weakness. She denied flushing, nausea, vomiting, diarrhea, polydypsia, polyuria, or vaginal bleeding. She denied any medication use other than iron sulfate. On examination, her sitting blood pressure was 140/100 mm Hg with a pulse of 92 beats/min; standing blood pressure was 120/80 mm Hg with a pulse of 176 beats/min. The remainder of the examination was unremarkable including skin turgor, cardiac, neurologic, rectal, and pelvic findings. She received 4 L of normal saline solution in the emergency room before admission.

Physical examination on admission to the medical floor showed a thin, black woman who was nervous but otherwise comfortable. Her temperature was 36.8° C, supine blood pressure 138/82 mm Hg with pulse of 76 beats/min, sitting blood pressure 122/84 mm Hg with pulse of 96 beats/min, and a standing pulse of 120 beats/min. The lungs were clear to auscultation. Cardiovascular examination showed a normal point of maximal impulse, jugular venous pressure of 6 cm, and normal S_1 and S_2 sounds with no appreciable murmur. The abdomen was normal, and digital rectal examination showed guaiac-negative stool. A pelvic examination was unremarkable with no vaginal discharge or bleeding.

Laboratory test samples drawn before saline solution infusion in the emergency room included hematocrit of 33%, white blood cell count of 6×10^9/L, a platelet count of 271×10^9/L, and a reticulocyte count of 3.9%. The erythrocyte sedimentation rate was 7 mm/hr. Serum electrolyte analysis showed the following levels: sodium, 138 mmol/L; potassium, 4.6 mmol/L; chloride, 103 mmol/L; bicarbonate, 23

From the Department of Medicine, University Hospitals of Cleveland, Case Western Reserve University.
Supported by a grant from the National Institutes of Health (K08 AG00387).
Received for publication April 25, 1990; accepted June 15, 1990.
Reprint requests: Scott L. Mader, MD, Geriatric CARE Center, 2074 Abington Road, Cleveland, OH 44106.
6/1/23227

mmol/L; and glucose, 4.33 mmol/L. A urine toxicology screen was negative. An electrocardiogram showed borderline sinus tachycardia; the chest x-ray film was normal.

The patient's hospital course was remarkable for continued orthostasis despite aggressive rehydration. The following tests were obtained and had normal results: pelvic ultrasonography, thyroid function tests, urine electrolytes and specific gravity, cosyntropin stimulation test, aldosterone, prolactin, luteinizing hormone, follicle-stimulating hormone, echocardiogram, and computerized tomography of the head. Autonomic function testing was performed on the fifth hospital day and is shown in Table I.

The patient was treated with fludrocortisone, 0.1 mg twice daily; atenolol, 50 mg daily, high-salt diet, elevation of the head of the bed with blocks, and was instructed to spend as much time as possible in the upright position. On this regimen her condition improved after 2 days and her weight increased approximately 3 kg over the next week. Follow-up blood pressure readings are shown in Table I. All medications were tapered after 1 month with no recurrence of symptoms and no change in orthostatic blood pressure parameters.

Table I. Autonomic function testing results

Condition	*Blood pressure (mm Hg)*	*Pulse (beats/min)*
Pretreatment		
Supine	122/79	102
Standing	121/93	136
Walking	112/88	136
Isometric handgrip	+11/+19	+20
Cold pressor	+10/+5	+10
Supine after standing	+30/+10	−46
After treatment		
Supine	124/80	80
Standing	114/78	88
Walking	117/77	93

All readings were taken with an automated blood pressure cuff (Physiocontrol Lifestat, Redmond, Wash.). Supine readings are the mean of the second and third readings after 5 minutes of supine rest. Standing values are after 60 seconds in the upright position. Walking values are values after 5 minutes of standing and 2 minutes of walking. Isometric handgrip is the maximum change from baseline during 3 minutes of 30% maximal handgrip. Cold pressor is the maximum change from baseline after 1 minute of hand immersion in ice water.

Comment

This patient appeared to have orthostatic intolerance as a result of prolonged blood loss after a spontaneous vaginal delivery. However, despite the appropriate corrective measures (saline solution infusion, blood transfusion), she continued to have orthostatic symptoms with prominent tachycardia. She responded to combination treatment with a high-salt diet, elevation of the head of the bed, fludrocortisone, and β-blockade. When her blood pressure normalized, all treatment measures were stopped and she remained asymptomatic.

This type of orthostatic hypotension (with prominent tachycardia and narrowing of the pulse pressure) has also been called hyperadrenergic orthostatic intolerance, sympathotonic orthostatic hypotension, irritable heart syndrome, vasoregulatory asthenia, hyperkinetic heart syndrome, or hyperdynamic β-adrenergic circulatory state.[1] The hallmark of this condition is normal autonomic function with a brisk tachycardia to upright posture and relative maintenance of diastolic pressure.

Hyperadrenergic orthostatic intolerance can be seen with many conditions, including cardiac disease (mitral valve prolapse, paroxysmal tachycardias, atrial myxoma, hypertrophic cardiomyopathy), deconditioning (prolonged recumbency or space travel), inadequate blood volume (dehydration, bleeding, renal salt wasting, adrenal insufficiency, pheochromocytoma, diuretic use), excessive pooling (large varicose veins, pregnancy, or sympathectomy), excessive vasodilation (fever, medications, hypokalemia, hyperbradykininism, hot bathing, strenuous exercise), and other miscellaneous causes (idiopathic, postviral, or during rapid weight loss).

The pathophysiologic characteristics of hyperadrenergic orthostatic intolerance are not understood. Several investigators[2, 3] have studied this syndrome; from the data there appear to be components of autonomic nervous system hyperresponsiveness, volume depletion, and excessive venous pooling. The initiating event in most patients is not known. In our patient it appears that the primary event was blood loss caused by retained placental products. An important factor may have been the extended blood loss over a 2-week period before definitive treatment to stop the bleeding. As a result of the decreased blood volume, compensatory sympathetic nervous system overactivity developed. At this point, further attempts to reverse volume state were unsuccessful because of vasoconstriction caused by the sympathetic nervous system overactivity. Except for standing tachycardia and orthostasis, observation of her clinical state would suggest that her volume status was normal because she was not thirsty, had normal skin turgor, and had normal jugular venous pressure. Only after combination treatment with volume expansion, elevation of the head of the bed, and β-blockade (to decrease sympathetic nervous system outflow) did her symptoms resolve. Use of clonidine may have been equally efficacious to β-adrenergic blockade.[2]

REFERENCES

1. Blomqvist CG. Orthostatic hypotension. Hypertension 1986;8:722-31.
2. Fouad FM, Tadena-Thome L, Bravo EL, Tarazi RC. Idiopathic hypovolemia. Ann Intern Med 1986;104:298-303.
3. Streeten DHP, Anderson GH, Richardson R, Thomas FD. Abnormal orthostatic changes in blood pressure and heart rate in subjects with intact sympathetic nervous function: evidence for excessive venous pooling. J Lab Clin Med 1988;111:326-35.

Cervicovaginal microflora and pregnancy outcome: Results of a double-blind, placebo-controlled trial of erythromycin treatment

James A. McGregor, MDCM,[a] Janice I. French, CNM, MS,[a] Ruth Richter, CNM, MSN,[a] Margo Vuchetich, BSN,[a] Vaundeen Bachus, BSN,[a] Kyung Seo, MD,[a] Sharon Hillier, PhD,[b] Franklyn N. Judson, MD,[c] John McFee, MD,[c] Judith Schoonmaker, PhD,[a] and James K. Todd, MD[d]

Denver, Colorado, and Seattle, Washington

Available information suggests that some instances of preterm birth or premature rupture of membranes are associated with clinically unrecognized infection and inflammation of the lower uterine segment, decidua, and fetal membranes. Various cervicovaginal microorganisms have been recovered from these sites. Many of these microorganisms produce factors that may lead to weakening of the fetal membranes, release of prostaglandins, or both. This study evaluated the presence of various lower genital tract microflora and bacterial conditions in 229 women enrolled in a double-blind, placebo-controlled trial of short-course erythromycin treatment at 26 to 30 weeks' gestation to prevent preterm birth. Demographic, obstetric, and microbiologic parameters were prospectively evaluated. Premature rupture of membranes occurred less frequently ($p < 0.01$) among women who received erythromycin (6%) versus placebo (15%). Preterm premature rupture of membranes also occurred less frequently, although not significantly ($p = 0.3$) in patients who received erythromycin (2%) versus placebo (5%). Erythromycin treatment significantly decreased the occurrence of premature rupture of membranes among women who were initially positive for *Chlamydia trachomatis* infection. Logistic regression analysis demonstrated that *C. trachomatis* ($p = 0.05$; odds ratio, 9), vaginal wash phospholipase C ($p = 0.08$; odds ratio, 6) and prior preterm birth ($p = 0.007$; odds ratio 17) were associated with increased risk of preterm birth. Bacterial vaginosis, *Mycoplasma hominis, Ureaplasma urealyticum* were not significantly associated with increased risk of preterm birth or preterm rupture of membranes. These findings support a role for selected lower genital tract microflora in preterm birth and premature rupture. Large controlled treatment trials of specific infections or conditions associated with preterm birth and premature rupture of membranes are required to confirm the value of antimicrobial treatments in prevention of microbial-associated preterm birth. (Am J Obstet Gynecol 1990;163:1580-91.)

Key words: Prematurity, premature rupture of membranes, infection, antibiotic, treatment, prevention

Gestational age and birth weight are the most important biologic determinants of an individual's chances for survival and healthy growth and development.[1] Approximately 300,000 infants are born in the United States each year with the biologic disadvantages of prematurity. Of these approximately 15% to 20% die in the newborn period.[1]

From the Department of Obstetrics and Gynecology, University of Colorado Health Sciences Center,[a] the Department of Obstetrics and Gynecology, University of Washington School of Medicine,[b] Denver Public Health and Hospitals and the Department of Medicine and Preventive Medicine, University of Colorado Health Sciences Center,[c] and the Department of Pediatrics, The Children's Hospital.[d]
Received for publication March 23, 1990; revised May 25, 1990; accepted July 27, 1990.
Reprint requests: James A. McGregor, MDCM, 4200 E. 9th Ave., Box B198, Denver, CO 80262.
6/1/24110

To be maximally effective in prevention of preterm birth, intervention strategies should be cause specific and based on understanding of pathophysiology. Much information indicates that infectious processes and resultant inflammation in and around the uteroplacental unit are involved in the pathogenesis of a proportion of women who suffer preterm labor, premature rupture of membranes, and premature birth.[2] Mechanisms by which cervicovaginal microflora and host inflammatory cells may act to mediate preterm birth require further elucidation. Cervicovaginal microorganisms may gain access to the lower uterine segment through the cervix and directly mediate infection involving the decidua, the chorioamnion, and possibly the placenta. Such infections may be initially unrecognized, and if uncontained, can progress to clinically apparent chorioamnionitis with intraamniotic fluid in-

fection and possible fetal sepsis.[3] Cervicovaginal microflora and host inflammatory cells may also mediate or promote preterm labor or preterm rupture of membranes through the release of various phospholipases and proteases.[4, 5]

Attempts to improve pregnancy outcome with antimicrobial treatment to eliminate specific lower urogenital tract microorganisms remain inconclusive but encouraging. Elder et al.[6] noted increased birth weight among newborns of women without bacteriuria who were treated antenatally with a 6-week course of tetracycline.[6] In an attempt to eliminate genital infections caused by *Ureaplasma urealyticum, Mycoplasma Hominis,* or both organisms among pregnant women, Kass et al.[7] and McCormack et al.[8] demonstrated increased birth weight among newborns of pregnant women who received erythromycin for up to 6 weeks during the third trimester. Neither study specifically discussed the occurrence of preterm premature rupture of membranes or the presence of other various genitourinary tract microflora. Recently Nugent et al. were unable to demonstrate benefits from antenatal erythromycin treatment in women colonized with *U. urealyticum* but not *M. hominis,* group B streptococcus, or *Chlamydia trachomatis* (Nugent RP, et al., Society for Epidemiologic Research Abstracts, 1989;929-30). In a study focused on women with group B streptococcal urinary tract colonization, Thomsen et al.[9] reported a reduction in the occurrence of premature rupture of membranes and preterm birth among women treated with penicillin. Among women hospitalized with preterm labor who received parenteral tocolytic therapy, several studies have shown mixed results of erythromycin or ampicillin treatment or a combination of the two drugs in prolonging pregnancy.[10, 11]

We undertook this study to prospectively evaluate associations of cervicovaginal microflora and selected lower genital tract microbe-associated factors with pregnancy outcomes. We used a short course of orally administered erythromycin to "probe" the relationship of these microbial factors or conditions with obstetric and neonatal outcomes including premature rupture of membranes, preterm premature rupture of membranes (<37 weeks' gestation), gestational length, birth weight, and newborn infection in a group of inner-city pregnant women.

Methods

From October 1985 to August 1988, 235 women between 26 and 30 weeks' gestation who attended publicly supported antenatal clinics in the Denver metropolitan area were enrolled in this prospective double-blind, placebo-controlled study. Prior evaluations of unselected women at these same inner-city sites showed rates of preterm birth between 16% to 20%, with the majority of these births occurring after 26 weeks' gestation (John McFee, MD, personal communication). In the studies by Kass et al.[7] and McCormack et al.,[8] only erythromycin given in the third (vs second) trimester of pregnancy was associated with increased birth weight. Therefore we enrolled and treated patients between 26 and 30 weeks' gestation.

Written informed consent was obtained as approved by the institutional human subjects committees. Women were excluded if they had a recognized cause for prematurity (multiple gestation, placenta previa, or cervical incompetence); if they had severe cardiac, renal, respiratory or immunologic diseases; or if they were allergic to erythromycin. Subsequently six women were excluded from analysis: four women were lost to follow-up after enrollment, one was treated for premature labor on the day of enrollment, and one woman experienced intrauterine fetal death at 30 weeks' gestation. The total evaluated sample included 229 women and their newborns.

Clinical evaluation. Women were enrolled by one of three experienced clinicians. Participants were interviewed to obtain demographic, social, behavioral, sexual, medical, and obstetric information. Specific information regarding urogenital symptoms was recorded.

A vaginal speculum examination was performed and characteristics of vaginal discharge (consistency, amount, color, odor) were recorded on standard forms. Samples were obtained as follows. One milliliter of saline solution was inoculated with a cotton swab containing vaginal fluid for microscopic examination for *Trichomonas vaginalis,* yeast pseudohyphae, and release of amine odor with addition of 10% potassium hydroxide "whiff test." This sample was then held at 4° C and evaluated in the laboratory for nonspecific protease activity using Azocoll substrate.[5] Vaginal fluid pH was assessed directly with Nitrazine paper (Squibb, Princeton, N.J.) and recorded as >4.5 or ≤4.5. An additional sample of vaginal fluid was allowed to air dry on a slide for Gram stain evaluation of bacterial vaginosis and *Mobiluncus* spp. morphotypes.[12]

Separate endocervical swabs were immediately inoculated onto a chocolate agar and a modified Thayer-Martin split plate for the recovery of *Neisseria gonorrhoeae* organisms, and into sucrose-phosphate glutamate transport media for recovery of *C. trachomatis.* Cervicovaginal swabs were inoculated onto 5% sheep blood agar (tryptic soy base, Remel, Denver) for recovery of group A streptococcus, group B streptococcus, *Staphylococcus aureus, Gardnerella vaginalis,* and yeast organisms. These microorganisms were identified with standard techniques. Additional vaginal swabs were inoculated into a standard mycoplasma medium for recovery of *M. hominis* and *U. urealyticum.* Cultures for *C. trachomatis, M. hominis,* and

U. urealyticum specimens were frozen at −70° C and processed in batches. After 48 hours' incubation at 35° C, inoculated cycloheximide-treated McCoy cell monolayers were treated with fluorescence-conjugated anti-chlamydia monoclonal antibody (Syva Microtrak, Syva Co., Palo Alto) and examined microscopically for the presence of stained inclusions. Negative cultures were blind passaged once and reprocessed. *M. hominis* and *U. urealyticum* organisms were cultured and identified with the Mycotrim GU Triphasic Culture System (Hana Biologics, Inc., Alameda, Calif.)

After microbial samples were obtained and vaginal pH was determined, the posterior vaginal fornix was irrigated with 4 ml of sterile Dulbecco's buffered saline solution and after standardized mixing was aspirated into a sterile syringe. The vaginal wash specimens were frozen immediately at −20° C, then frozen at −70° C within 4 hours of collection. Wash samples were evaluated in batches for nonspecific protease,[5] phospholipase C,[13] and total protein (Biorad, Richmond, Calif.).

Proline aminopeptidase was quantified in these samples by a modification of the method of Thomasen et al.[14] Vaginal samples were incubated for 4 hours with proline nitroanilide (0.2 gm/100 ml Tris buffer) at 37° C, 6% carbon dioxide. Samples were read at 450 nm and compared with standard leucine aminopeptidase for quantitation.

Treatment and follow-up. After initial examination the women were given a sealed bottle containing 21 tablets and were instructed to take one tablet three times a day for 1 week, and to record when they took the tablet on a medication diary. Treatment was prepared and randomized by means of a computer-generated random number list by The Upjohn Company (Kalamazoo). Women received identical-appearing treatment bottles and tablets that were either erythromycin base (E-Mycin, Upjohn) 333 mg, or placebo. Patients continued routine prenatal care after enrollment in the study.

Two to four weeks after enrollment the women were seen by one of the investigators during a routine prenatal visit; diaries and bottles were collected and unused pills were counted. Women were questioned regarding side effects, sexual activity, and signs and symptoms of vaginitis before repeat vaginal sampling.

Only positive *N. gonorrhoeae* cultures were reported to health care providers. Except for one patient with *N. gonorrhoeae* infection, sexual partners of patients were not referred for evaluation and treatment. Maternal and newborn medical records were reviewed to obtain additional information on antenatal, intrapartum, and postpartum maternal course and the newborn course through 3 months of age. If records were unavailable the data were obtained by telephone calls and written correspondence to private physicians and patients.

Definitions. Gestational age was determined before data analysis and based on best clinical criteria, which included last menstrual period, fetal growth, and ultrasonographic measurements, and newborn gestational age assessment.[15] Preterm labor was defined as regular uterine contractions (two or more in 10 minutes) treated with hospitalization and at least one subcutaneous injection or intravenous infusion of tocolytic agent followed by oral tocolytic therapy, or labor that resulted in a preterm birth. Patients with preterm labor that followed preterm premature rupture of membranes were not counted as preterm labor patients. Premature rupture of membranes was defined as rupture of membranes at least 1 hour before the onset of uterine contractions. Preterm premature rupture of membranes was defined as rupture of membranes before labor (as above) before 37 weeks' gestation. Preterm birth was considered as birth before completion of 37 weeks' gestation. Infants who weighed <2500 gm at birth were considered low birth weight.

Data analysis. Data were summarized by use of descriptive statistics. Statistical significance of treatment group differences and other univariate associations were tested with χ^2 or Fisher's exact test (two-tailed). Continuous data were analyzed with Student's t test. When appropriate, the relative risk and 95% confidence intervals were calculated. Findings from initial microbiologic tests were analyzed for associations with pregnancy outcomes. To control the effects of multiple independent variables on the development of preterm birth, low birth weight, and premature rupture of membranes, logistic regression analyses were performed with the SAS LOGIST program (SAS Institute Inc., Cary, N.C.) on a VAX computer. Multivariate analysis was not attempted for the dependent variables preterm premature rupture of membranes or preterm birth without prior premature rupture of membranes because of the small number of women with these outcomes. Alpha was set at 0.05 for both univariate and multivariate analysis. The Breslow-Day test for homogeneity of results of odd ratios was used to confirm significance as appropriate.

Results

The demographic aspects of study women are described in Table I. There were no differences between demographic, historical, or behavioral characteristics for the 119 women randomized to receive erythromycin and the 110 women who received placebo. Use of "crack" or cocaine was reported by five women who received erythromycin and two who received placebo. Each of these women was delivered at term.

Reported signs and symptoms of vaginitis and characteristics of vaginal discharge were similar between study groups. Approximately 5% of patients in each group noted vaginal irritation, itching, or burning at

Table I. Demographic, historical, and behavioral characteristics for women treated with erythromycin and placebo

Characteristic	*Erythromycin (N = 119)*	*Placebo (N = 110)*
Demographic		
Maternal age (yr)		
Mean ± SD	23.0 ± 4.3	23.2 ± 4.2
Range	13-37	16-34
Ethnicity		
White	45 (37.8%)	43 (39.1%)
Black	42 (35.3%)	42 (38.2%)
Hispanic	30 (25.2%)	21 (19.1%)
Other	2 (1.6%)	4 (3.6%)
Unmarried	66 (55.5%)	60 (54.5%)
Historical		
Gravidity		
Mean ± SD	2.9 ± 1.8	2.8 ± 1.6
Range	1-11	1-8
Primigravid	29 (24.4%)	30 (27.3%)
Past preterm birth†	15 (12.6%)	12 (10.9%)
Past sexually transmitted disease‡	61 (53%)	57 (54.3%)
Past pelvic inflammatory disease	24 (20.9%)	20 (19.2%)
Behavioral		
Smoking (>10 cigarettes/day)	29 (24.4%)	36 (32.7%)
Alcohol (any)	25 (21.0%)	30 (27.3%)
Street drug use (any)	23 (19.3%)	14 (12.7%)

*One or more prior births <37 weeks' gestation.

†History prior to this pregnancy of any of the following: pelvic inflammatory disease, gonorrhea, chlamydia, trichomonas, syphilis, herpes simplex virus, genital warts.

enrollment. No patient was diagnosed with vaginitis by her regular health care provider on the day of study enrollment.

Recovery rates for the test microorganisms and vaginal substances were similar at study enrollment among the patients randomized to receive erythromycin or placebo, except that women randomized to the placebo group more often had vaginal pH >4.5 (Table II).

Follow-up examination was performed approximately 1 month ($\bar{x}$ = 27 days) after study enrollment for 75 (63%) women who were treated with erythromycin and 81 women (74%) who were given placebo. Vaginal recovery of *M. hominis* among women who received erythromycin was 24% at intake and 12% after treatment (Table II). However, among the nine women treated with erythromycin who were positive for *M. hominis* at entry and available for reexamination, seven remained positive at follow-up. Detection of *C. trachomatis* and *Mobiluncus* spp. morphotypes among women who were initially positive was decreased after erythromycin treatment, but not significantly (p = 0.1, data not shown). Detection of group B streptococcus, bacterial vaginosis, *U. urealyticum, Staphylococcus aureus, G. vaginalis,* yeast species, proline aminopeptidase, levels of vaginal phospholipase C, nonspecific protease, and vaginal pH >4.5 did not differ from pretreatment levels or prevalence after erythromycin treatment (Table II).

Antenatal complications. Various medical problems were experienced by 19 (16%) patients in the erythromycin group and 11 (10%) patients in the placebo group (p = 0.2). These complications were well controlled and not implicated in preterm birth, except for one patient in the placebo group who was diagnosed with systemic lupus erythematosus (preterm delivery with premature rupture of membranes) and one patient treated with erythromycin who required antihypertensive and asthma-related medications (delivery at term).

Twenty-five percent of patients in the erythromycin group and 16% of patients in the placebo group reported antenatal vaginal bleeding (p = 0.1). One patient in the placebo group was diagnosed with a low-lying placenta that resolved; she was delivered at term without further complications. An additional patient in the erythromycin group was diagnosed with placenta previa after study enrollment. This patient was seen with complaints of preterm premature rupture of membranes at 33 weeks' gestation; she was delivered at 36 weeks by cesarean section after demonstration of fetal lung maturity.

Seven women in the erythromycin group (6%) and five women in the placebo group (4%) were followed up for gestational diabetes. Polyhydramnios developed in two women in each treatment group, and three women in the erythromycin group (2%) and one (1%) patient who received placebo had oligohydramnios. Seven (6%) patients in the erythromycin group and five (4%) patients in the placebo group were followed

Table II. Pretreatment and posttreatment microorganisms and virulence factors detected for erythromycin and placebo groups

Test	*Intake*		*Posttreatment**	
	Erythromycin (N = 119)	*Placebo (N = 110)*	*Erythromycin (N = 75)*	*Placebo (N = 81)*
N. gonorrhoeae	1 (1%)†	0	0	0
C. trachomatis	13 (12%)	13 (12%)	7 (10%)	9 (12%)
U. urealyticum	74 (76%)	82 (84%)	42 (64%)	58 (84%)
M. hominis	24 (24%)	17 (18%)	8 (12%)	12 (17%)
Bacterial vaginosis	32 (27%)	25 (23%)	19 (26%)	21 (27%)
Mobiluncus species	15 (13%)	14 (13%)	9 (12%)	12 (16%)
G. vaginalis	27 (23%)	32 (29%)	20 (27%)	20 (26%)
T. vaginalis	4 (4%)	5 (5%)	1 (1%)	3 (4%)
S. aureus	8 (7%)	4 (4%)	2 (3%)	3 (4%)
Group B streptococcus	6 (5%)	1 (1%)	6 (8%)	2 (3%)
Group A streptococcus	1 (1%)	1 (1%)	1 (1%)	1 (1%)
Yeast species	42 (35%)	44 (40%)	22 (29%)	18 (24%)
Vaginal pH > 4.5	23 (21%)	37 (37%)‡	20 (28%)	22 (29%)
Proline aminopeptidase	36 (32%)	37 (36%)	20 (32%)	20 (31%)
Phospholipase C	7 (6%)	9 (9%)	3 (5%)	7 (10%)
Nonspecific protease	26 (24%)	32 (30%)	17 (25%)	21 (28%)

*There were no differences in the overall frequency of recovered agents or related factors among patients sampled before and after study treatments.

†Number and percent positive.

‡$p = 0.01$; relative risk, 0.6; 95% confidence interval, 0.4 to 0.9.

up for intrauterine growth retardation. Pregnancy-induced hypertension was diagnosed among four (3%) erythromycin patients and four (4%) placebo patients antenatally. Each was delivered at term.

Two patients experienced intrauterine fetal death, one of undetermined cause at 30 weeks in a patient in the placebo group and one fetus with Down's syndrome at 42 weeks in a women in the erythromycin treatment group. The patient with the 30-week-gestation fetal death was not considered in analysis for preterm outcome variables; all other patients with concurrent or intercurrent antenatal medical or obstetric problems were included in the analysis.

Sixty-six percent of the erythromycin group and 69% of the placebo group received nonprotocol antimicrobial therapy during antenatal care. No patient received antibiotics within 2 weeks of study treatment. The preponderance of treatments occurred before study entry (first trimester: 21%; second trimester: 40%; third trimester: 37%). Vaginitis was the most common reason for antimicrobial treatment. Approximately 20% of both groups received treatment during pregnancy for a single urinary tract infection.

Compliance. The mean number of study pills taken differed between groups (erythromycin, 15.9 ± 7.8; placebo, 17.8 ± 5.9; $p = 0.05$). Only 73% of women randomized to receive erythromycin and 84% of women who received placebo completed 4 or more days of study treatment ($p = 0.04$). Side effects, including nausea, vomiting, and diarrhea, were reported significantly ($p < 0.05$) more often among women who received erythromycin (nausea: erythromycin = 27%; placebo = 13%; $p = 0.01$; vomiting: erythromycin = 13%; placebo = 4%; $p = 0.02$; diarrhea: erythromycin = 5%; placebo = 0; $p = 0.03$).

Pregnancy outcome. Spontaneous vaginal delivery occurred among 80% of patients in the erythromycin group and 71% of placebo patients. Cesarean section delivery was performed for 8% and 12% of patients who received erythromycin and placebo, respectively. Assisted vaginal delivery occurred in the remaining patients.

There were no overall differences in the occurrence of preterm labor, preterm birth, or low-birth-weight infants among treatment groups when the data were analyzed by univariate analysis. The mean gestational age at delivery was 39.3 ± 1.9 weeks among patients in the erythromycin group and 39.2 ± 1.6 weeks for those who received placebo. Mean birth weight was 3132 ± 588 gm among erythromycin patients and 3099 ± 548 gm among placebo patients ($p = 0.7$). Further analysis showed no difference in gestational age or birth weight at delivery even for those patients who had completed at least 4 days of study treatment [gestational age at delivery: erythromycin ($N = 87$), 39.3 ± 1.7 weeks; placebo ($N = 93$), 39.4 ± 1.6 weeks; birth weight: erythromycin, 3191 gm; placebo, 3161 gm] or those who completed the full 1 week of study treatment [gestational age at delivery: erythromycin ($N = 57$), 39.2 ± 1.7 weeks; placebo ($N = 60$), 39.4 ± 1.6

weeks; birth weight: erythromycin, 3195 gm, placebo, 3180 gm].

Two patients in the erythromycin group (2%) and three (3%) patients in the placebo group were treated for clinically diagnosed chorioamnionitis. Postpartum endometritis occurred among six (6%) patients in the erythromycin group and eight (8%) in the placebo group. There were no significant differences between study groups for any neonatal outcome evaluated.

Premature rupture of membranes. Rupture of membranes before labor occurred significantly more often among women who received placebo (16%) compared with those who received erythromycin (6%), ($p = 0.01$; relative risk, 0.4; 95% confidence interval, 0.2 to 0.8). Preterm premature rupture of membranes occurred among seven women and was also decreased among women in the erythromycin group, although this failed to reach statistical significance (erythromycin, 2%; placebo, 5%; $p = 0.3$; relative risk, 0.4; 95% confidence interval, 0.1 to 1.8).

Trends were noted between selected medical factors and premature rupture of membranes; more women who experienced premature rupture of membranes gave a history of prior sexually transmitted disease (erythromycin, 15%; placebo, 7%; $p = 0.06$, relative risk, 2; 95% confidence interval, 1.0 to 4.9) and women who experience preterm premature rupture of membranes more often had a history of a single prior abortion (erythromycin, 7%; placebo, 2%; $p = 0.08$; relative risk, 4; 95% confidence interval, 0.9 to 14.8). None of the other demographic, behavioral or other medical problems analyzed were significantly associated with increased risk of premature rupture of membranes or preterm premature rupture of membranes.

Overall, premature rupture of membranes (any gestational age) occurred significantly more often among women from whom *C. trachomatis* was recovered at study enrollment ($p = 0.03$; relative risk, 2.5; confidence interval, 1.1 to 5.7) and also among those with vaginal pH >4.5 ($p = 0.03$; relative risk, 2.5; 95% confidence interval, 1.1 to 5.5). Women with *U. urealyticum* infection experienced premature rupture of membranes more frequently; however, this did not reach statistical significance ($p = 0.08$, relative risk, 5.0, 95% confidence interval, 0.9 to 27.8).

To test for effects of erythromycin treatment on pregnancy outcomes, data were stratified according to the presence of susceptible microorganisms or abnormal vaginal conditions. None of the 13 women treated with erythromycin from whom *C. trachomatis* was initially recovered experienced premature rupture of membranes. Conversely, six of 12 women with positive *C. trachomatis* cultures who received (50%) placebo experienced premature rupture of membranes ($p = 0.03$, relative risk, 0.4; 95% confidence interval, 0.2 to 0.8). The Breslow-Day test for homogeneity of results of odds ratios confirmed this significant difference ($p = 0.03$). Among women from whom *U. urealyticum* was recovered, the occurrence of premature rupture of membranes was significantly less in those who received erythromycin (erythromycin, 7%; placebo, 18%, $p = 0.03$; relative risk, 0.4; 95% confidence interval, 0.1 to 0.9); among women who were negative for *U. urealyticum*, only a single patient in the placebo group experienced premature rupture of membranes (Breslow-Day test, NS). Among the women with vaginal pH >4.5, women in the erythromycin group experienced premature rupture of membranes less often, although not significantly (erythromycin, 4%; placebo, 24%; $p = 0.07$; relative risk, 0.2; 95% confidence interval, 0.03 to 0.96).

Preterm premature rupture of membranes occurred more often among women whose initial cultures were positive for *S. aureus* (erythromycin, 2/12 [17%]; placebo, 5/209 [2%]); ($p = 0.04$; relative risk, 7; 95% confidence interval, 1.8 to 28.4). Women with positive findings at enrollment for *C. trachomatis*, *T. vaginalis*, group B streptococcus, yeast species, phospholipase C, positive whiff test, and pH >4.5 also experienced preterm premature rupture of membranes more frequently; however, these associations failed to reach statistical significance.

Preterm birth. Preterm birth occurred among eight (7%) women in the erythromycin group and nine (8%) women who received placebo. History of a prior preterm birth was the only historical, demographic, or behavioral variable significantly associated with preterm birth in the current pregnancy ($p = 0.002$; relative risk, 4; 95% confidence interval, 1.7 to 9.9). Recovery of *C. trachomatis* was significantly associated with increased risk of preterm birth ($p = 0.03$; relative risk, 3; 95% confidence interval, 1.2 to 8.1). A positive whiff test was also significantly associated with increased risk of preterm birth ($p = 0.04$; relative risk, 3; 95% confidence interval, 1.1 to 7.6) as was the presence of both pH >4.5 and a positive whiff test ($p = 0.03$; relative risk, 4; 95% confidence interval, 1.3 to 10.6). Vaginal pH >4.5 alone was not significantly associated with increased risk of preterm birth ($p = 0.2$; relative risk, 2; 95% confidence interval, 0.7 to 5.1). Overall, bacterial vaginosis (determined by Gram stain) and positive proline aminopeptidase were not associated with preterm birth. Preterm birth occurred more frequently, but not significantly, among the small number of women with *T. vaginalis*, *S. aureus*, and increased phospholipase C found at initial examination.

Preterm birth occurred before completion of 34 weeks' gestation in five patients. *C. trachomatis* ($p = 0.01$; relative risk, 11; 95% confidence interval, 2.8 to 46), *M. hominis* ($p = 0.008$; relative risk, 26; 95% con-

Table III. Cervicovaginal findings and selected risk factors for five patients with delivery ≤34 weeks

	Patient no. 1	*Patient no. 55*	*Patient no. 109*	*Patient no. 119*	*Patient no. 141*
Prior preterm birth	−	+	+	−	−
Prior abortion	+	+	−	+	−
C. trachomatis	−	+	+	+	−
T. vaginalis	−	−	+	−	−
Bacterial vaginosis	−	−	−	+	+
Proline aminopeptidase	−	+	+	+	+
pH >4.5	ND	+	+	+	+
Whiff test	−	+	+	+	+
Mobiluncus species	−	−	−	+	−
U. urealyticum	ND	+	U	+	+
M. hominis	ND	+	U	+	+

ND, Not done; *U,* unevaluable specimen.

fidence interval, 1.4 to 494), proline aminopeptidase ($p = 0.04$; relative risk, 8; confidence interval 95%, 1.2 to 48), pH >4.5 ($p = 0.07$; relative risk, 8; 95% confidence interval, 1.1 to 50), and positive whiff test ($p = 0.02$; relative risk, 11; 95% confidence interval, 2.5 to 44) were significantly associated with increased risk of birth ≤34 weeks' gestation. Of note, four of these five women had findings of multiple microbiologic factors thought to be associated with increased risk of prematurity (Table III).

Preterm birth in the absence of premature rupture of membranes occurred among nine women and significantly more often among women with both vaginal pH >4.5 and positive whiff test ($p = 0.03$; relative risk, 6; 95% confidence interval, 1.6 to 19.5). Similarly, preterm birth without prior rupture of membranes occurred more frequently, but not significantly, among women who were positive for *C. trachomatis* ($p = 0.07$), *T. vaginalis, M. hominis,* proline aminopeptidase ($p = 0.09$), whiff test, and phospholipase C.

Multivariate analysis. Logistic regression analysis was performed to control for the effects of multiple independent variables on the occurrence of preterm birth, low birth weight, or premature rupture of membranes (Table IV). As a preliminary step, a logistic regression analysis was performed to identify significant independent variables from among maternal demographic, behavioral, and past medical history variables that were predictive of preterm birth, low birth weight, or premature rupture of membranes, Among these independent variables (maternal age, ethnic group, marital status, gravidity, prior abortion, prior preterm birth, use of alcohol, street drugs, smoking, and history of pelvic inflammatory disease), only prior preterm birth was significantly associated with preterm birth and low birth weight ($p = 0.001$).

Second, on the basis of results of previously published studies or theoretical associations, another model was constructed that included bacteriologic findings (*C. trachomatis, T. vaginalis, U. urealyticum, M. hominis, S. aureus,* bacterial vaginosis), vaginal enzymes (high vaginal nonspecific protease, phospholipase C), vaginal pH >4.5, treatment group, use of other antenatal antibiotics, and prior preterm birth. In this second model (Table IV), *C. trachomatis* ($p = 0.05$; OR, 9), phospholipase C ($p = 0.08$; OR, 6) and prior preterm birth ($p < 0.01$; OR, 17) were associated with preterm birth. After adjusting for the effects of the other independent variables in the model, erythromycin treatment was associated with decreased risk of preterm birth ($p = 0.06$; OR, 0.1).

A final model was created that included only the independent variables that were statistically significant or bordered on statistical significance (*C. trachomatis,* phospholipase C, prior preterm birth, and study treatment). In this final model, *C. trachomatis* ($p = 0.02$; OR, 4) and prior preterm birth ($p = 0.009$; OR, 5) were the only variables associated with increased risk of preterm birth.

In similar analyses, low birth weight (<2500 gm) was positively associated with prior preterm birth ($p = 0.003$, OR, 8) and negatively associated with erythromycin treatment ($p = 0.02$, OR, 0.2); and erythromycin treatment was associated with decreased risk of premature rupture of membranes ($p = 0.01$; OR, 0.1) (Table IV).

Because of the small number of women who experienced preterm premature rupture of membranes, preterm birth ≤34 weeks' gestation, and preterm birth in the absence of prior premature rupture of membranes, these outcomes were not evaluated with multivariate techniques.

In this study, cervical infection with *C. trachomatis* accounted for 20% of the preterm birth (population attributable risk). Such calculations are probably oversimplifications because an individual mother's host susceptibility and the presence of different microorganisms are not considered.

Table IV. Results of multivariate analysis

Dependent variable	*Independent variable*	*Significant independent variable*	*Error of beta*	*beta*	*p Value*	*R*
Preterm birth	Demographic, historical, medical factors*	Prior preterm birth	1.7	0.56	0.002	0.25
Preterm birth	Prior preterm birth	Prior preterm birth	2.9	1.06	0.007	0.3
	Cervicovaginal microorganisms*	*C. trachomatis*	2.2	1.14	0.05	
		Phospholipase C	1.8	1.06	0.08	
	Erythromycin treatment	Erythromycin treatment	−2.7	1.41	0.06	
Preterm birth	Prior preterm birth	Prior preterm birth	1.5	0.58	0.009	0.22
	C. trachomatis Phospholipase C Erythromycin treatment	*C. trachomatis*	1.5	0.62	0.02	
Birth weight <2500 gm	Prior preterm birth	Prior preterm birth	2.0	0.69	0.003	0.08
	Cervicovaginal microorganisms* Erythromycin treatment	Erythromycin treatment	−1.7	0.72	0.02	
Premature rupture of membranes	Prior preterm birth	Erythromycin treatment	−2.4	0.96	0.01	0.08
	Cervicovaginal microorganisms* Erythromycin treatment					

*See text.

Comment

We prospectively studied 229 pregnant women and their singleton infants to identify and measure associations of various demographic, obstetric, behavioral, and reproductive tract microbiologic–related factors with subsequent maternal and neonatal outcomes. We further used a short, defined course of oral enteric-coated erythromycin base to more dynamically assess possible microbiologic relationships and pregnancy outcomes. Study patients were inner-city women typical of those treated at public teaching hospitals. There were no significant differences between the drug- or placebo-treated groups in demographic, historical, obstetric, behavioral, or microbiologic-related parameters at study entry, except for an increased number of women with high vaginal pH among placebo patients. Findings were typical of similar populations.[10, 15-17] Rates of intercurrent, antenatal, intrapartum, and neonatal complications also appeared typical of this population.

The relatively low incidence (7% to 8%) of preterm birth among enrolled women was probably a result of selection biases imposed by entry criteria. Overall rates (16% to 20%) of preterm birth for the clinics studied did not change during the course of this study. Perhaps because of increasing interest in possible associations of infection and preterm birth, approximately two thirds of the women in both groups received nonstudy antimicrobials from antenatal care providers. It might be expected that additional antibiotics would obscure detection of study treatment effects. However, when nonstudy antimicrobial therapy was analyzed by logistic regression, no association with preterm birth, low birth weight, or premature rupture of membranes was noted.

Neither the women who received erythromycin nor women treated with placebo tolerated study medication well; only 73% of the erythromycin and 84% of the group placebo completed at least 4 days of medication. Significantly, more women who took erythromycin complained of nausea and vomiting and took fewer tablets. This relatively poor compliance may have been caused by (1) general reticence to take medications during pregnancy, (2) inadequate understanding or identification with overall study goals, (3) lack of a specific treatment indication (i.e., treatment of an acknowledged pathogen), or (4) other factors. Other than

nausea, vomiting, and diarrhea, there were no other recognized complications of erythromycin treatment among women or newborns.

Univariate analysis of intake findings and subsequent preterm birth or premature rupture of membranes showed that occurrence of a prior preterm birth was the only nonmicrobiologic factor linked with preterm delivery. There were no demographic, historical, or behavioral findings significantly associated with premature rupture of membranes or preterm premature rupture of membranes. Toth et al.[16] have suggested that persistent effects of prior endometrial infection may mediate recurrent preterm birth. In our study, a past history of any sexually transmitted disease was linked to occurrence of premature rupture of membranes ($p = 0.06$), but not preterm birth or preterm premature rupture of membranes ($p = 0.1$). History of prior pelvic infection or sexually transmitted disease was commonly reported among our patients (Table I), which may have mitigated any specific effect of prior reproductive tract infection. In addition to possibly persisting reproductive tract infections, it is likely that unrecognized intrinsic or metabolic factors associated with preterm birth may also persist in subsequent pregnancies. Elucidation and elimination of such persistent factors could reduce preterm birth significantly in women at risk.

The numbers and percentages of subjects with various cervicovaginal microorganisms are similar to those found in previous studies of our patients and other urban populations using comparable procedures.[10, 17] The overall inability of study drug to eliminate the microorganisms tested for is likely explained by (1) the lack of erythromycin activity at lower vaginal pH,[18] (2) short duration of treatment (lack of compliance), or (3) lack of treatment of sexual partners.

Cervical infection with *C. trachomatis* was significantly associated with increased risks of premature rupture of membranes and preterm birth, and was recovered more frequently, although not significantly, among women who experienced preterm birth without premature rupture of membranes and preterm premature rupture of membranes. These findings support those of Martin et al.,[19] Harrison et al.,[20] Berman et al.,[21] and Sweet et al.,[22] who also noted associations of *C. trachomatis* genital tract infection with either preterm birth or low birth weight and premature rupture of membranes.

The latter three studies noted increased risks of preterm birth, low birth weight, and preterm premature rupture of membranes only among women who demonstrated antichlamydial IgM antibody, rather than among all women infected with *C. trachomatis*.[20-22] The authors suggested that the presence of specific antichlamydial IgM denoted either recent infection or "invasive" upper genital tract chlamydial infection.[20-22] Subclinical endometritis occurs commonly (>30%) among nonpregnant women with chlamydial cervicitis[23] and may also occur during pregnancy.

Vaginal presence of *U. urealyticum* was associated with subsequent premature rupture of membranes ($p = 0.08$) but not preterm birth ($p = 0.3$). Others have noted increased *U. urealyticum* genital colonization or placental infection among patients with premature rupture of membranes, preterm birth, or both.[24, 25] *U. urealyticum* was the most common microorganism recovered between layers of amniochorion in patients who were delivered before 37 weeks' gestation in a case-controlled study by Hillier et al.[25] Although intrauterine infection with *U. urealyticum* is well described, the frequency of genital infection among women with uncomplicated pregnancy (up to 85%) suggests a complex relationship between *U. urealyticum* infection and abnormal reproductive outcomes. Quinn et al.[26] suggested that certain *U. ureaplasma* serotypes are more likely to be associated with preterm birth. Many strains of *U. urealyticum* produce phospholipase A_1, A_2, and C as well as IgA proteases in vitro.[27] Among our patients, recovery of *U. urealyticum* was the only microbial association noted with high vaginal concentrations of phospholipase C (data not shown). Hillier et al.[25] further suggested that interactions between *U. urealyticum* and other vaginal conditions or microflora may be important in determining the virulence of ureaplasmas.

Vaginal pH >4.5 (detected here by Nitrazine paper) was associated with premature rupture of membranes ($p = 0.03$; relative risk = 2.5) by means of univariate analysis, but not when the presence of other microbiologic conditions were controlled by logistic regression. An association between increased vaginal pH and abnormal pregnancy outcomes was noted by both Minkoff et al.[28] and Ernest et al.[29] Whereas Ernest measured vaginal pH with a special pH meter, both the study by Minkoff et al. and our study used widely available but less accurate pH paper. Vaginal pH itself may alter the lower reproductive tract milieu and, possibly, directly affect processes involved in labor or membrane rupture by some as yet undefined mechanism. Both Minkoff et al.[28] and Ernest et al.[29] suggest that increased vaginal pH is more likely a marker for the presence of abnormal genital tract microfloras, including the presence of bacterial vaginosis, *T. vaginalis*, or both.

In this study bacterial vaginosis (Gram stain diagnosis) was not significantly associated with preterm birth or preterm membrane rupture. However, bacterial vaginosis, presumptively diagnosed by the presence of proline aminopeptidase, was found in four of five women who were delivered at <34 weeks' gestation ($p = 0.04$) (Table III). Similarly, in a prospective study of 233 women, Minkoff et al.[17] were unable to dem-

onstrate a statistically significant association between clinical findings suggesting of bacterial vaginosis and preterm birth or preterm premature rupture of membranes. Minkoff et al.[17] did note increased risk of preterm birth and premature rupture of membranes among women from whom *Bacteroides* species and *M. hominis* (microorganisms commonly associated with bacterial vaginosis) were recovered.

Conversely, other studies found increased risk of preterm labor, birth, or membrane rupture among women with bacterial vaginosis.[30, 31] Differences in the methods used to detect bacterial vaginosis, study design, populations studied, sample size, or true lack of effort could account for such differences.

As previously shown by Toth et al.,[16] vaginal recovery of *S. aureus* was associated with preterm rupture of membranes and preterm birth. Although less frequently isolated than other bacteria, strains of *S. aureus* recovered from the vagina may be powerful producers of collagenases and phospholipases A_2 and C as well as a variety of other possible virulence factors.[4, 5]

Although taken only for relatively few days ($\bar{x}$ = 5 days) by patients in this study, univariate analysis showed that erythromycin treatment was associated with reduced premature rupture of membranes (p = 0.01; relative risk = 0.4; 95% confidence interval = 0.2 to 0.8). Multivariate analysis (Table IV) showed that erythromycin treatment was associated with reduced low birth weight (p = 0.02), premature rupture of membranes (p = 0.01), and possibly preterm birth (p = 0.06). Prior controlled trials of antibiotic treatment during pregnancy used lengthy courses of either tetracycline, erythromycin, or clindamycin, given orally for as long as 6 weeks.[6-8] Elder et al.[6] administered tetracycline (122 patients) versus placebo (109 patients) to inner-city women from 1963 to 1965. They noted longer duration of pregnancy (tetracycline: $\bar{x}$ = 39.1 weeks; placebo: $\bar{x}$ = 38.1 weeks; χ^2 = 2.4) and greater birth weight (tetracycline: $\bar{x}$ = 115.6 ounces; placebo: $\bar{x}$ = 110.8 ounces; χ^2 = 1.7). Premature rupture of membranes was not further defined but occurred in 9.9% of women who received tetracycline versus 12.5% of women treated with placebo (χ^2 = 1.9).

Similar investigations from 1971 to 1980 were done by Kass et al.[7] to evaluate erythromycin or clindamycin administered orally for 6 weeks in comparison with placebo among women with genital tract *M. hominis* or *U. urealyticum* infections. These investigators also noted incomplete compliance; fewer than half the patients completed the treatment protocols. A trend for increased birth weight was noted only if (1) mothers took the study antibiotic for 6 weeks, (2) they demonstrated ≥fourfold increase in titer to *U. urealyticum,* and (3) if the 6-week erythromycin treatment was continued in the third trimester (second trimester; low birth weight: erythromycin, 7%; placebo, 8% versus third trimester; low birth weight: erythromycin, 3%; placebo, 12%; p = 0.063). Despite such intensive treatment, vaginal ureaplasmas or mycoplasmas persisted at follow-up examination, as in our study. Kass et al.[7] suggested that the antibiotic action was directed toward "reducing invasion from the vaginal source," rather than eliminating carriage of vaginal mycoplasmas.[7] Subsequently, Quinn et al.[32] noted that placentas from women who received either erythromycin or doxycycline were rarely infected with mycoplasmas.

In further analysis, McCormack et al.[8] noted that of the various treatments, only clindamycin taken for at least 3 weeks was associated with reduced recovery of *M. hominis;* neither erythromycin preparation nor clindamycin altered recovery of *U. urealyticum.* By means of a one-tailed analysis, McCormack et al. again showed significant increases in birth weight only among women who were deemed to have completed 6 weeks of erythromycin treatment begun in the third trimester (erythromycin, 3330.7 gm; placebo, 3187 gm; p = 0.042). McCormack et al.[8] again suggested that the effects of erythromycin may not be specific for mycoplasmas and that "interactive effects of other agents such as chlamydia, anaerobic bacteria and other agents" should be investigated. As an example of such possible interactive effects, Table III demonstrates the frequency of identified genital infection and conditions among the five women in our study who were delivered at ≤34 weeks' gestation. Four of five had multiple, easily detectable, and potentially treatable vaginal infections or conditions.

How treatment with erythromycin or similar antibiotics might reduce perinatal morbidity remains unknown, but several possibilities exist. As noted, erythromycin possesses antimicrobial activity against a number of microorganisms associated with premature rupture of membranes: *C. trachomatis,* group B streptococcus, *S. aureus,* and mycoplasmas. Although it is relatively inactive at vaginal pH, erythromycin is active at serum pH and achieves uterine tissue concentration approximating maternal serum.[18] Clindamycin, a related antibiotic, also achieves higher concentrations in fetal membranes than in maternal serum.[33] Preliminary information suggests erythromycin is also relatively concentrated in membranes (R. Bowden, PhD, personal communication). Increased concentrations as well as relatively enhanced activity of these antibiotics within amniochorion and surrounding tissue likely inhibit growth or metabolism of susceptible microorganisms. As suggested by McCormack et al.,[8] effective endometrial level concentrations of erythromycin could also reduce ascent of cervicovaginal microorganisms.

Other factors may also play roles in reduction of perinatal morbidity. Much work shows that erythro-

mycin and other antibiotics that interfere with bacterial protein synthesis can suppress or eliminate release of bacterial virulence factors (such as fetal membrane-damaging proteases) even at subinhibitory concentrations.[34] In vitro studies show that both subminimal inhibitory concentrations and supraminimal inhibitory concentrations of erythromycin eliminate bacterial protease induced weakening of fetal membrane preparations and that erythromycin does not alter measures of membrane strength.[34] Correspondingly, in this study effects of erythromycin appeared linked to the presence of specific, erythromycin-susceptible cervicovaginal microflora.

The results of our study support suggestions that maternal genital tract infections or associated inflammatory changes play possibly preventive roles in the pathogenesis of preterm birth in an important number of women.

Short-course treatment with erythromycin base was associated with reduced risks of premature rupture of membranes and low birth weight and possible preterm birth ($p = 0.06$) (multivariate analysis). Erythromycin appeared safe but was not well tolerated, thus empiric erythromycin treatment is not yet recommended. Controlled studies to evaluate the efficacy and safety of focused antimicrobial treatments during pregnancy are under way. Understanding of the mechanisms through which maternal genital tract infection mediate preterm birth awaits further basic and applied microbiologic and immunologic study.

REFERENCES

1. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82-90.
2. McGregor JA. Prevention of preterm birth: new initiatives based on microbial-host interactions. Obstet Gynecol Surv 1988;43(1):1-14.
3. Hameed C, Tejani N, Verma UL, Archbald F. Silent chorioamnionitis as a cause of pretern labor refractory to tocolytic therapy. Am J Obstet Gynecol 1984;149:726-30.
4. Bejar R, Curbelo V, Davis C, Gluck L. Premature labor. II. Bacterial sources of phospholipase. Obstet Gynecol 1981;57:479-82.
5. Schoonmaker JN, Lawellin DW, Lunt B, McGregor JA. Bacteria and inflammatory cells reduce chorioamniotic membrane integrity and tensile strength. Obstet Gynecol 1989;74:590-6.
6. Elder HA, Santamarina BAG, Smith S, Kass EH. The natural history of asymptomatic bacteriuria during pregnancy: the effect of tetracycline on the clinical course and the outcome of pregnancy. Am J Obstet Gynecol 1971; 111:441-62.
7. Kass EH, McCormack WM, Lin J-S, Rosner B, Munoz A. Genital mycoplasmas as a cause of excess premature delivery. Trans Assoc Am Physicians 1981;94:261-6.
8. McCormack WM, Rosner B, Lee Y-H, Munoz A, Charles D, Kass EH. Effects on birth weight of erythromycin treatment of pregnant women. Obstet Gynecol 1987;69: 202-7.
9. Thomsen AC, Morup L, Hansen KB. Antibiotic elimination of group B streptococci in urine in prevention of preterm labor. Lancet 1987;1:591-3.
10. McGregor JA, French JI, Reller LB, Todd JK, Makowski EL. Adjunctive erythromycin treatment for idiopathic preterm labor: results of a randomized, double-blinded, placebo-controlled trial. Am J Obstet Gynecol 1986;154: 98-103.
11. Newton ER, Dinsmoor MJ, Gibbs RS. A randomized, blinded, placebo-controlled trial of antibiotics in idiopathic preterm labor. Obstet Gynecol 1989;74:562-6.
12. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct Gram's stain of vaginal fluid. J Clin Microbiol 1983;18:170-7.
13. Berka RM, Gray GL, Vasil ML. Studies of phospholipase C (heat-labile hemolysin) in *Pseudomonas aeruginosa*. Infect Immun 1981;34:1071-4.
14. Thomasen JL, Gelbart SM, Wilcoski MT, Peterson AK, Jilly BJ, Hamilton PR. Proline aminopeptidase activity as a rapid diagnostic test to confirm bacterial vaginosis. Obstet Gynecol 1988;71:607-11.
15. Ross MG, Hobel CJ, Bragonier JR, Bear MB, Bemis RL. A simplified risk-scoring system for prematurity. Am J Perinatol 1986;3(4):339-44.
16. Toth M, Witkin SS, Ledger W, Thaler H. The role of infection in the etiology of preterm birth. Obstet Gynecol 1988;71:723-6.
17. Minkoff H, Grunebaum AN, Schwartz RH, et al. Risk factors for prematurity and premature rupture of membranes: a prospective study of vaginal flora in pregnancy. Am J Obstet Gynecol 1984;150:965-72.
18. Durfee MA, Forsyth PS, Hale JA, Holmes KK. Ineffectiveness of erythromycin for treatment of *Haemophilus vaginalis*-associated vaginitis: possible relationship to acidity of vaginal secretions. Antimicrob Agents Chemother 1979;16:635-7.
19. Martin DH, Koutsky L, Eschenbach DA, et al. Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infection. JAMA 1982; 247:1585-8.
20. Harrison HR, Alexander ER, Weinstein L, Lewis M, Nash M, Sim DA. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy. JAMA 1983;250: 1721-7.
21. Berman SM, Harrison HR, Boyce WT, Hoffner WJJ, Lewis M, Arthur JB. Low birth weight, prematurity, and postpartum endometritis. JAMA 1987;257:1189-94.
22. Sweet RL, Landers DV, Walker C, Schachter J. *Chlamydia trachomatis* infection and pregnancy outcome. Am J Obstet Gynecol 1987;156:824-33.
23. Paavonen J, Kiviat N, Brunham RC, et al. Prevalence and manifestations of endometritis among women with cervicitis. Am J Obstet Gynecol 1985;152:280-6.
24. Kundsin RB, Driscoll SG, Monson RR, Yeh C, Blano SA, Cochran WD. Association of *Ureaplasma urealyticum* in the placenta with perinatal morbidity and mortality. N Engl J Med 1984;310:941-5.
25. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-controlled study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1988;319:972-8.
26. Quinn PA, Shewchuk AB, Shuber J, et al. Serologic evidence of *Ureaplasma urealyticum* in women with spontaneous pregnancy loss. Am J Obstet Gynecol 1983;145: 245-50.
27. DeSilva NS, Quinn PA. Endogenous activity of phospholipases A and C in *Ureaplasma urealyticum*. J Clin Microbiol 1986;23:354-9.
28. Minkoff H, Grunebaum A, Feldman J, Cumming SM, McCormack WM. Relationship of vaginal pH and Papanicolaou smear results to vaginal flora and pregnancy outcome. Int J Gynaecol Obstet 1987;25:17-23.
29. Ernest JM, Meis PJ, Moore ML, Swain M. Vaginal pH: a

marker of premature rupture of the membranes. Obstet Gynecol 1989;74:734-8.
30. Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA 1986;256:1899-1903.
31. Martius J, Krohn MA, Hillier SL, Stamm WE, Holmes KK, Eschenbach DA. Relationships of vaginal *Lactobacillus* species, cervical *Chlamydia trachomatis* and bacterial vaginosis to preterm birth. Obstet Gynecol 1988;71:89-95.
32. Quinn PA, Shewchuk AB, Shuber J, et al. Efficacy of antibiotic therapy in preventing spontaneous pregnancy loss among couples colonized with genital mycoplasmas. AM J OBSTET GYNECOL 1983;145:239-44.
33. Gilstrap LC, Bawdon RE, Burris J. Antibiotic concentration in maternal blood, cord blood, and placental membranes in chorioamnionitis. Obstet Gynecol 1988; 72:124-5.
34. McGregor JA, Schoonmaker JN, Lunt BD, Lawellin DW. Antibiotic inhibition of bacterially-induced fetal membrane weakening. Obstet Gynecol 1990;76:124-8.

Vitamin E levels in premenstrual syndrome

C. James Chuong, MD, MPH, Earl B. Dawson, PhD, and Edward R. Smith, PhD
Galveston, Texas

To determine whether changes in peripheral vitamin E levels are associated with symptoms of premenstrual syndrome, 10 patients with premenstrual syndrome and 10 control subjects were studied. They gave blood at 2- or 3-day intervals throughout three menstrual cycles. The vitamin E was measured by fluorometry after hexane extraction and luteinizing hormone was measured by radioimmunoassay. In the control group, vitamin E values were 854 ± 45 μg/dl (mean ± SE) during the luteal phase and 925 ± 55 μg/dl during the follicular phase. No significant changes were noted between the two values. In the patients with premenstrual syndrome, the values were 849 ± 53 μg/dl during the luteal phase, which was not significantly different from 858 ± 48 μg/dl during the follicular phase. No significant changes were noted between the control group and the patients with premenstrual syndrome in either the luteal or the follicular phase. Vitamin E deficiency in patients with premenstrual syndrome was not shown by peripheral vitamin E levels in our study. (AM J OBSTET GYNECOL 1990;163:1591-5.)

Key words: Vitamin E, premenstrual syndrome

Many theories have been proposed to understand the pathophysiology of premenstrual syndrome (PMS) in the hope of finding an effective treatment.[1] The Biskinds[2] were the first to propose the role of nutritional factors in PMS. Over the past few years, nutritional supplements have been widely used as a treatment of PMS.[3-6] The use of these supplements is based on the assumption that patients with PMS consume more refined sugar, refined carbohydrates, and dairy products, and less vitamins such as vitamin E than do normal women.[7] The deficiency of vitamin E was suggested to be related to premenstrual symptoms.[8] Excessive and aberrant prostaglandin synthesis have been implicated in the pathogenesis of PMS,[9] and there was some evidence suggesting that vitamin E could modulate the production of prostaglandin. Supplemental vitamin E blocks the age-related increase in prostaglandin E in mice,[10] whereas it reduces the release of arachidonic acid from phospholipids.[11]

There are no valid conclusions on the efficacy of vitamin E in the treatment of PMS, although some investigators reported that vitamin E was an effective treatment for breast tenderness and other PMS symptoms.[12, 13] However, it was noted that the placebo and vitamin E were not administered in a cross-over fashion for each patient, and vitamin E deficiency was never shown in these patients when compared with control subjects. Serum vitamin E levels may assist in the assessment of a vitamin E deficiency in patients with PMS and serve as a guideline before and during vitamin E treatment. Therefore the objective of this study is to determine whether changes in peripheral vitamin E levels are associated with PMS symptoms.

From the Department of Obstetrics and Gynecology, University of Texas Medical Branch.
Supported in part by the University of Texas Medical Branch Intramural Grant Award 18511-622010.
Received for publication May 11, 1990; revised July 20, 1990; accepted July 27, 1990.
Reprint requests: C. James Chuong, MD, MPH, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.
6/1/24143

Material and methods

The study was approved by the Institutional Review Board of the University of Texas Medical Branch, Galveston. Ten patients, 24 to 39 years of age, in general good health with regular menses for at least six previous cycles were studied. All were interviewed by the same investigator (C. J. C.) for detailed medical history. All were from a middle-class population in southeast Texas. Interview on the diet history and daily intake of various nutrients did not reveal evidence of extraordinary food intake. Results were normal for physical examinations by either the investigator or the referral physicians within 6 months before the study began. They were in generally good health, and no significant medical conditions from history or on physical examination were noted. They had no history of psychiatric disorders, as determined by the schedule for affective disorders and schizophrenia—lifetime interview.

Before enrollment the patients were instructed to start charting the daily diary on the first day of the menses throughout the entire menstrual cycle after the initial visit and to qualitatively measure premenstrual complaints. The daily diary was designed by us to follow the diagnosis guidelines established by the National Institute of Mental Health. Each patient documented within the last half of the cycle at least three of the 18 symptoms, with at least one psychologic and one somatic symptom for at least one preceding menstrual cycle. The 18 symptoms included irritability; tension or anxiety; mood swings; emotional lability; restlessness; decreased concentration; depression; aggression; poor coordination; craving for sweet or salty food; lethargy; generalized swelling; breast tenderness; abdominal bloating; swelling of the face, hands, or feet, weight gain; headache; and change in bowel habits. Patients were eliminated from the study if the symptoms were present in the first half of the cycle or if the symptoms persisted for more than 2 days after the onset of menses.

Each subject was also instructed to complete the menstrual distress questionnaire[14] on days 7 and 25 of the same menstrual cycle. They also needed to complete the basal body temperature (BBT) chart during the same cycle. All of them showed a biphasic pattern with evidence of ovulation and adequate luteal phase. The menstrual distress questionnaire was described in previous studies.[14] Women whose scores on the questionnaire were >80 on day 7 or <95 on day 25 of the menstrual cycle were excluded from the study. With these entry criteria, the mean score (± SE) on the menstrual distress questionnaire was 55.2 ± 2.3 on day 7 and 125.7 ± 8.2 on day 25.

Ten female volunteers from among our institution's employees served as the control group. They were in general good health and had regular predictable menses. None of them had premenstrual symptoms or severe dysmenorrhea. Subjects were excluded if they had taken any drugs or medication including vitamin or nutritional supplements during the 8 weeks before the study. They were also asked to keep a daily diary and to complete the menstrual distress questionnaire and the BBT chart in the same fashion as the patient group. None of them showed significant premenstrual symptoms on the daily diary. They all showed a biphasic pattern and adequate luteal phase on the BBT chart. The mean score (± SE) on the menstrual distress questionnaire before the study was 53.1 ± 2.6 on day 7 and 51.5 ± 1.1 on day 25.

Informed consent was obtained from all the participants. After an overnight fast the blood samples for vitamin E, luteinizing hormone (LH), and follicle stimulating hormone (FSH) assays were drawn between 8 AM and 10 AM. Before the blood was collected the women sat quietly for 30 minutes. They gave blood at 2- or 3-day intervals, starting on day 1 of the menstrual cycle throughout three cycles. The patients and controls were instructed to complete the menstrual distress questionnaire on days 7 and 25 of the menstrual cycle for three cycles. They were also asked to complete the BBT chart during the same three cycles.

Ten milliliters of blood was collected, permitted to clot, and centrifuged at 1000 *g* for 15 minutes at 24° C. The serum was stored at −20° C until analyzed. The determination of vitamin E levels was by measurement of the ultraviolet fluorescence at 340 nm on activation at 295 nm, the absorption peak for pure α-tocopherol in hexane.[15,16] All glassware used in the procedure was acid-washed (5% nitric acid, rinsed with reagent-grade water, and air-dried). Two milliliters each of reagent-grade water (Milli-Q, Millipore, Bedford, Mass.) and absolute ethanol (redistilled at 82° C) were added with vortexing to denature the serum protein and release α-tocopherol. The free α-tocopherol was extracted by vortexing the serum homogenate with pure hexane (redistilled at 85° C, Fisher Scientific, Houston, Tex.) and centrifugation at 900 *g* for 5 minutes at 24° C. The hexane phase of each tube was transferred by glass pipette to 1 cm quartz cuvettes and the fluorescence measured with a Turner 430 spectrofluorometer (Sequoia-Turner, Palo Alto, Calif.). The serum α-tocopherol levels were determined by the comparison of the fluorescence of the serum hexane extract with the fluorescence of known levels of pure dl-α-tocopherol (Sigma Chemical Co., St. Louis). The results were expressed as μg/dl. The concentrations of standard dl-α-tocopherol solutions in absolute ethanol were from 200 to 2000 μg/dl in 100 μg increments and the fluorescence was linear throughout this range. The purity of the α-tocopherol standard was confirmed by spectral analysis, and the absorption peak of maximum

Table I. Age, body weight, marital status, and reproductive and menstrual histories of patients with PMS and control subjects

	Control (n = 10)	*PMS (n = 10)*
Age (yr)		
Median	30	32
Range	23-37	24-39
Body weight		
Average (% of IBW)*	107	105
Range (% of IBW)*	84-124	81-126
No. of obese subjects†	1 (124% of IBW)*	2 (120% and 126% of IBW)*
Marital status		
Single	5	3
Married	5	5
Divorced	0	2
Reproductive history		
Nulliparous	6	5
Parous	4	5
Menstrual history, median		
Menarch (yr)	12.0	13.5
Interval (days)	27.0	28.0
Length (days)	5.0	5.0
Dysmenorrhea		
None	2	1
Mild	4	3
Moderate	3	4
Severe	1	2

From Chuong CJ, Dawson EB, Smith ER. Vitamin A levels in premenstrual syndrome. Fertil Steril 1990;54:643. Reproduced with permission of the publisher, The American Fertility Society.

IBW, Ideal body weight.

*According to the table of the Metropolitan Life Insurance Company.

†Obesity is defined as body weight 20% greater than IBW.

fluorescence was observed at 295 nm excitation with 340 nm emission. A recovery study of 0.3 ml test aliquots of 50% serum pool and 100 μg/dl increments of dl-α-tocopherol in absolute ethanol from 200 through 2000 μg/dl resulted in 94% recovery of the added vitamin.

LH and FSH were measured by double-antibody radioimmunoassay. All reagents except the standards were obtained from the National Hormone and Pituitary Program (Baltimore, Md.). LH and FSH standards were obtained from the World Health Organization. The intraassay coefficient of variation was 7.3% for LH and 6.8% for FSH, whereas the interassay variations were 12.3% and 15% for LH and FSH, respectively.

All samples from the same patient were run in duplicate in the same assay for vitamin E, LH, and FSH.

The day of LH surge was considered as the midpoint of the cycle and was designated day 0. All other measurements were expressed as the number of days before or after the LH surge. The hormonal measurement during the follicular phase (day LH −14 to −3) of each month for each subject was the mean of all data during the follicular phases of each month. The mean of the three months' values for each subject was then obtained. The hormonal measurement during the luteal phase (day LH +3 to +14) was calculated in a similar fashion. The two-way analysis of variance (ANOVA) with the use of general linear models from SAS[17] was used to test for significant intragroup changes of vitamin E levels between the follicular and luteal phases. Comparisons between the control group and the group with PMS during the follicular and luteal phases were also made by ANOVA. The differences in the menstrual distress questionnaire scores were determined by the two-sample or paired-sample Student *t* test. The level of significance was $p < 0.05$.

Results

There were no differences in age, body weight, marital status, reproductive history, menstrual history, or degree of dysmenorrhea between the patient and control groups (Table I). The mean duration of PMS symptoms in the patient group was 60.5 months.

To participate in the study, the menstrual distress questionnaire score on day 7 had to be <80. The questionnaire score on day 7 before the study for the group with PMS was not significantly different from that of control subjects (55.2 ± 2.3 vs 53.1 ± 2.6, mean ± SE). The large difference in menstrual distress questionnaire score on day 25 (125.7 ± 8.2 for the group with PMS and 51.5 ± 1.1 for the control group) was expected, on the basis of the criteria for inclusion in

Table II. Mean vitamin E levels (μg/dl) over three menstrual cycles of patients with PMS and control subjects

	Control group		*PMS group*	
Subject	*F*	*L*	*F*	*L*
1	1238 (14)	850 (12)	701 (15)	662 (14)
2	852 (12)	886 (13)	560 (10)	600 (8)
3	720 (13)	957 (14)	904 (10)	1072 (15)
4	877 (14)	748 (13)	919 (15)	969 (12)
5	735 (11)	789 (14)	763 (14)	700 (14)
6	892 (13)	672 (10)	1052 (12)	998 (14)
7	1049 (12)	1140 (11)	967 (13)	935 (11)
8	1102 (15)	901 (8)	878 (15)	847 (12)
9	811 (15)	723 (13)	948 (12)	928 (12)
10	976 (16)	878 (13)	889 (15)	780 (15)
Mean ± SE	925 ± 55	854 ± 45	769 ± 143	849 ± 53

F, Follicular phase; *L*, luteal phase; numbers in parentheses are number of observations.

this study. A similar pattern of score changes were noted in each PMS and control subject each month during the study period. The mean of the 3 months' scores for the patients with PMS was 53.1 ± 1.9 during the follicular phase and 120.7 ± 6.2 during the luteal phase. For the control group, the mean was 49.8 ± 2.6 during the follicular phase and 52.8 ± 2.4 during the luteal phase.

All the participants showed a biphasic BBT pattern each month, suggesting ovulation and adequate luteal phase. The LH and FSH levels are shown in their characteristic patterns, with the day of the LH peak designated as day LH 0. The LH peak reached 92.3 ± 12.5 mIU/ml (mean ± SE), whereas FSH was 24.2 ± 5.3 mIU/ml on day LH 0. In the control group, vitamin E values were 854 ± 45 μg/dl (mean ± SE) during the luteal phase, and 925 ± 55 μg/dl during the follicular phase. No significant changes were noted between the two values. In the patients, the values were 849 ± 53 μg/dl during the luteal phase, which was not significantly different from 858 ± 48 μg/dl during the follicular phase. No significant changes were noted between the control group and the patients in either the luteal or the follicular phase (Table II).

Comment

It was hypothesized that vitamin E exerted its clinical effects on PMS symptoms through regulation of aberrant prostaglandin synthesis.[13] This was determined on the basis of the observation that there was an inhibitory effect of vitamin E on the release of arachidonic acid from its storage pool, which decreased the availability of prostaglandin $F_{2\alpha}$ precursors. The effect of vitamin E on the PMS symptoms was also hypothesized to be related to its modulatory effects, either directly or indirectly, on neurotransmitters in the central nervous system. Vitamin E supplementation blocked decreases γ-aminobutyric acid induced by prostaglandin precursors.[18] Radiolabeled vitamin E crossed the blood-brain barrier into several areas in the central nervous system in rat.[19] Vitamin E deficiency was associated with decreases in α-adrenergic binding sites in the central nervous system and decreases in choline acetyltransferase.[20]

However, vitamin E deficiency in PMS patients was not shown by peripheral vitamin E levels in our study. Our findings cannot explain the beneficial effect of vitamin E in PMS patients in previous double-blind studies.[12, 13] Those patients were assumed to have vitamin E deficiency before the treatment was given. The dosages of vitamin E administered to these patients ranged from 150 to 600 IU/day, which was 500% to 2000% of the U. S. recommended daily allowance for adults. We suggest that the response to vitamin E in these studies was a result of a pharmacologic response rather than correction of a deficiency state.

Another explanation for this discrepancy might be that vitamin E levels in the peripheral blood do not parallel those in the central nervous system. It remains possible that the bioavailability of vitamin E in the central nervous system, which is related to the activities of several neurotransmitters, could decrease during the luteal phase in some patients with PMS. As a result, the premenstrual symptoms occur. However, these changes in vitamin E levels in the central nervous system may not be reflected in the peripheral blood levels.

The procedures we used to measure serum vitamin E levels were described by Duggan[15] followed by Hansen and Warwick.[16] The same method has been used by several investigators.[21-23] In one study[22] The lower vitamin E levels in the peripheral blood of subjects with sickle cell anemia increased to the values similar to that of normal subjects after they were treated with vitamin E. It would be interesting to see whether our patients

with PMS will have increases of vitamin E levels in the peripheral blood and whether they will have decreases of symptoms after the vitamin E is supplemented.

Because of the rigorous initial entry criteria, such as at least a 2-month medication-free (including any form of vitamin or nutritional supplement) interval before testing, and the requirement of blood drawing at 2- or 3-day intervals for 3 months when no treatment could be given, the sample size in our study was limited. Yet these subjects would be expected to show vitamin E deficiency during the luteal phase if the deficiency is related to PMS symptoms, and can be shown in the peripheral circulation.

Mira et al.[24] measured several vitamin and trace element levels in the serum of patients with PMS on two occasions (premenstrual and postmenstrual) in 1 month and found no evidence for nutritional deficiencies including vitamin E. We now confirm and enlarge this previous observation. Further clarification of the role of vitamin E in central neurotransmitter activities and the relationship between the central and peripheral levels of vitamin E is needed. Until these issues are addressed, vitamin E supplementation can only be considered as an empirical therapy for PMS.

We thank Ms. Jo Ann Rabb for excellent editorial assistance in the preparation of the manuscript, as well as Elaine Brown, Michael Harris, William A. Harris, BA, and Sheila Jones for technical assistance.

REFERENCES

1. Reid RL, Yen SSC. Premenstrual syndrome. Am J Obstet Gynecol 1981;139:85-104.
2. Biskind MS, Biskind GR, Biskind LH. Nutritional deficiency in the etiology of menorrhagia, metrorrhagia, cystic mastitis, and premenstrual tension. Surg Gynecol Obstet 1944;78:49.
3. Abraham GE, Rumley RE. Role of nutrition in managing the premenstrual tension syndromes. J Reprod Med 1987;32:405-22.
4. Abraham GE. Management of the premenstrual tension syndrome: rationale for a nutritional approach. In: Bland J, ed. A year in nutritional medicine. New Haven, Connecticut: Keats Publishing, 1986:144.
5. Stewart A. Clinical and biochemical effects of nutritional supplementation on the premenstrual syndrome. J Reprod Med 1987;32:435-41.
6. Kendall KE, Schnurr PP. The effects of vitamin B6 supplementation on premenstrual symptoms. Obstet Gynecol 1987;70:145-9.
7. Goei GS, Ralston JL, Abraham GE. Dietary patterns of patients with PMT. J Appl Nutr 1982;34:4-11.
8. London RS, Sundaram GS, Murphy L, et al. Evaluation and treatment of breast symptoms in patients with the premenstrual syndrome. J Reprod Med 1983;28:503-8.
9. Jakubowicz DL. The significance of prostaglandins in the premenstrual syndrome. In: Taylor RW, ed. Premenstrual syndrome. London: Medical New-Tribune, 1983.
10. Meydani SN, Meydani M, Verdon CP, et al. Vitamin E supplementation suppresses prostaglandin E1(2) synthesis and enhances the immune response of aged mice. Mech Ageing Dev 1986;34:191-201.
11. Panganamala RV, Cornwall DG. The effects of vitamin E on arachidonic acid metabolism. Ann NY Acad Sci 1982;396:376.
12. London RS, Sundaram GS, Murphy L, et al. The effect of alpha-tocopherol on premenstrual symptomatology: a double-blind study. J Am Coll Nutr 1983;2:115-22.
13. London RS, Murphy L, Kitlowski KE, Reynolds MA. Efficacy of alpha-tocopherol in the treatment of premenstrual syndrome. J Reprod Med 1987;32:400-4.
14. Moos RH. The development of a menstrual distress questionnaire. Psychosom Med 1968;30:853-67.
15. Duggan DE. Spectrofluorometric determination of tocopherols. Arch Biochem Biophys 1959;84:116.
16. Hansen LG, Warwick WJ. A fluorometric micro-method for serum tocopherol. Am J Clin Path 1966;46:133-8.
17. SAS user guide. Cary, North Carolina: SAS Institute, 1986.
18. Chan PH, Kerlan R, Fishman RA. Reductions of gamma-amniobutyric acid and glutamate uptake and (Na+ + K+)-ATPase activity in brain slices and synaptosomes by arachidonic acid. J Neurochem 1983;40:309-16.
19. Vatassery GT, Angerhofer CK, Knox CA, et al. Concentrations of vitamin E in various neuroanatomical regions and subcellular fractions, and the uptake of vitamin E by specific areas of rat brain. Biochem Acta 1984;792:118-22.
20. Noda Y, McGeer PL, McGeer EG. Lipid peroxides in brain during aging and vitamin E deficiency: possible relations to changes in neurotransmitter indices. Neurobiol Aging 1982;3:173-8.
21. Leonard PJ, Doyle E, Harrington W. Levels of vitamin E in the plasma of newborn infants and of the mothers. Am J Clin Nutr 1974;25:480-4.
22. Machlin L. Plasma levels of tocopherol in sickle cell anemia subjects. Am J Clin Nutr 1979;32:1359-62.
23. Haga P, EK J, Kran S. Plasma tocopherol levels and vitamin E/B-liproprotein relationships during pregnancy and in cord blood. Am J Clin Nutr 1982;36:1200-4.
24. Mira M, Stewart PM, Abraham SF. Vitamin and trace element status in premenstrual syndrome. Am J Clin Nutr 1988;47:636-41.

Intrauterine *Listeria* infection: Prenatal diagnosis by biophysical assessment and amniocentesis

Robert I. Liner, MD
San Francisco, California

Diagnosis of intrauterine listeriosis and fetal salvage were facilitated by amniocentesis in a nonlaboring patient with fever and decreased fetal movement at 35 weeks' gestation. (AM J OBSTET GYNECOL 1990;163:1596-7.)

Key words: Amniocentesis, biophysical assessment, listeriosis

Maternal listeriosis is known to be associated with a minimum of clinical symptoms but a high degree of perinatal mortality and morbidity. We report a case in which biophysical assessment for decreased fetal movement prompted amniocentesis in a nonlaboring, minimally ill patient at 35 weeks' gestation. Microscopic analysis of the fluid facilitated a prenatal diagnosis of fetal sepsis as a result of *Listeria*, allowing immediate initiation of antibiotic therapy, cesarean delivery, and fetal salvage.

Case report

Our patient was a 30-year-old woman, gravida 4, para 0, at 35 weeks' gestation. Three days after a reactive nonstress test—performed because of an episode of vaginal bleeding with a low-lying placenta—she called to report an absence of fetal movement over the past 12 hours. She had not bled again but reported malaise, increased flatulence, and mild gas pains. She had not noticed fever, chills, or contractions.

On examination she seemed anxious but not ill. She was surprised to hear that she had a fever of 38.6° C. The pharynx was slightly erythematous. The abdomen was benign. The uterus was soft, nontender, and nonirritable. There were no contractions. Fetal heart activity was monitored at a rate of 160 beats/min without decelerations. Variability was present but diminished. Her cervix was long and closed; the vertex was ballotable.

The report of absent fetal movement and the decreased variability led to biophysical assessment. Despite rapid infusion of a liter of dextrose in 5% lactated Ringer's solution, the fetus appeared inert; two points were given for fluid and two for cardiac activity for a score of 4.

Unexplained maternal fever and verified lack of fetal activity suggested the possibility of sepsis, so the biophysical assessment was followed by amniocentesis. We obtained clear fluid with a striking yellowish green color. An immediate microscopic examination of stained and prestained fluid disclosed 4+ Gram-positive rods with a tumbling motility characteristic of *Listeria monocytogenes*. Because of ampicillin allergy, the patient was started on intravenous erythromycin while preparations were made for operative delivery. The intensive care nursery was advised of the probability of fetal listeriosis and, because of the evidence of fetal distress, general anesthesia was induced and the patient was promptly delivered of the infant.

Approximately 1 hour after the mother's hospital admission, a shocky 2200 gm female was delivered with Apgar scores of 2 and 4 at 1 and 5 minutes, respectively. The hematocrit was unremarkable. Cord blood was acidotic: pH (venous), 7.21; P_{CO_2}, 52; and P_{O_2}, 23. A dopamine drip was required to maintain the infant's blood pressure for the first 18 hours after birth. Ventilatory support was required for 28 hours. Cultures of maternal blood, amniotic fluid, fetal blood, and fetal gastric aspirate were positive for *Listeria;* the fetal cerebrospinal fluid was culture negative. The placenta, in addition to acute chorioamnionitis, had microabscesses typically associated with *Listeria* placentitis.

The mother had a benign postoperative course. She was discharged on postoperative day 4. The infant received a 2-week course of ampicillin combined with 1 week of gentamicin and has been entirely well since discharge 2 weeks after delivery.

Comment

Listeriosis in human beings occurs primarily in circumstances of altered immunity, including pregnancy. Because maternal symptoms are vague and nonspecific, they are easily dismissed as influenza. For that reason, diagnosis is generally not made until the patient is in labor or, more often, until after the delivery of a sick neonate. In such cases of congenital listeriosis, the mortality has been reported to be as high as 71%.[1]

Among the few cases in which maternal *Listeria* infection has been specifically diagnosed and treated prenatally, the organism has most often been recovered

From the Mount Zion Hospital and Medical Center.
Received for publication March 5, 1990; accepted June 2, 1990.
Reprints not available.
6/1/22874

from maternal blood or from the cervix. Amniocentesis has been recommended in midtrimester in the evaluation of otherwise unexplained maternal fever. A recent case report documents a third-trimester case of congenital listeriosis in which amniocentesis was performed in the context of uterine irritability and a threatening cervix at 32 weeks' gestation.[2] But we are not aware of any reported case in which a prenatal diagnosis of *Listeria* sepsis has resulted from amniocentesis prompted by lack of fetal movement. Our case serves to illustrate the point that reduced fetal activity may be a sign of amnionitis and fetal sepsis even in the absence of either contractions or other signs to suggest uterine infection.

REFERENCES

1. Evans JR, Allen AC, Bortolussi R, Issekutz TB, Stinson DA. Follow-up study of survivors of fetal and early onset neonatal listeriosis. Clin Invest Med 1984;7:329-34.
2. Grundy HO, Dannar C. Listeria monocytogenes—a cause of unexplained death in low-risk patients? Missouri Med 1988;85:149-50.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Pregnancy outcomes among mothers infected with human immunodeficiency virus and uninfected control subjects

Howard L. Minkoff, MD,[a] Cassandra Henderson, MD,[b] Hermann Mendez, MD,[c] Mitchell H. Gail, MD, PhD,[d] Susan Holman, RN, MS,[e] Anne Willoughby, MD,[f] James J. Goedett, MD,[g] Arye Rubinstein, MD,[h] Pamela Stratton, MD,[e] Judith H. Walsh,[i] and Sheldon H. Landesman, MD[e]

Brooklyn and Bronx, New York, and Bethesda, Maryland

Between June 26, 1985, and Feb. 24, 1989, 101 seropositive pregnant women and 129 seronegative pregnant women from the same prenatal clinics in Brooklyn and the Bronx were recruited into a prospective study of human immunodeficiency virus infection in pregnant women and their offspring. This report details the course of pregnancy and short-term neonatal outcomes of 91 seropositive women and 126 seronegative women who gave birth during the study period. Seropositive mothers were significantly more likely to have sexually transmitted diseases (17.6% vs 7.1%, $p = 0.017$) and medical complications (43.0% vs 25%, $p = 0.006$) during pregnancy. No other obstetric complications (e.g., chorioamnionitis, endometritis, toxemia, or placental problems) were associated with serologic status. After controlling for confounding variables (drug use, tobacco use, age of mother, and clinic), we found that the mother's serologic status was not significantly associated with birth weight, gestational age, head circumference, or Apgar scores among live infants. For example, after adjustment on confounders we found that children born to seropositive mothers weighed about 7 gm more than children of seronegative mothers (95% confidence interval, −180 to 194 gm). We conclude that in this population human immunodeficiency virus infection has little demonstrable impact on the status at birth of live neonates. (Am J Obstet Gynecol 1990;163:1598-604.)

Key words: Human immunodeficiency virus, pregnancy outcomes, sexually transmitted diseases

Anonymous newborn surveillance programs have provided estimates of human immunodeficiency virus (HIV) serologic prevalence among pregnant women in various parts of the United States.[1-3] In New York state, for example, where one of the most comprehensive programs has been instituted, 0.66% of neonates are born with passively transferred maternal HIV antibody in the blood.[1] Programs in Massachusetts have revealed that 0.75% of neonates in inner-city hospitals are similarly exposed to HIV.[2] Studies in more than 44 states, similar to the investigations conducted in New York and Massachusetts, result in estimates that >5000 HIV-infected women give birth annually in the United States. Coincident with the recognition of this problem, prenatal HIV counseling and testing programs have been recommended by a variety of agencies (including the Centers for Disease Control[4] and the American College of Obstetricians and Gynecologists[5]) and have been instituted in many prenatal centers.[6, 7] Information necessary to appropriately counsel and manage women identified as seropositive includes data on the short-term outcome of pregnancy. To date, published results from studies analyzing the effect of HIV infection on pregnancy outcomes (aside from those performed in Africa where some unique factors present in these populations influence birth outcomes) have been limited by failure to control for potential significant confounders, such as alcohol and tobacco use, and

From the Departments of Obstetrics and Gynecology,[a] Pediatrics,[c] and Internal Medicine[e] at State University of New York Health Science Center at Brooklyn; the Departments of Obstetrics and Gynecology[b] and Pediatrics[h] at Albert Einstein College of Medicine, Bronx; the Division of Children, Adolescents and Mothers with AIDS, National Institute of Child Health and Human Development,[f] and the Epidemiologic Methods Section[d] and the Viral Epidemiology Section,[g] National Cancer Institute and Westat.[i]

Supported by National Cancer Institute and National Institute of Child Health and Human Development contract No. N01-CP-61013, New York State AIDS Institute contract No. #C001720, and National Institutes of Health Clinical Research Center grant No. RR00318.

Received for publication January 10, 1990; revised May 10, 1990; accepted June 29, 1990.

Reprint requests: Howard Minkoff, MD, SUNY-Health Science Center at Brooklyn, Department of Obstetrics and Gynecology, Box 24-450 Clarkson Ave., Brooklyn, NY 11203-2098.

6/1/23591

Table I. Study patients giving birth by clinic, HIV status, and prenatal drug use

Clinic	*HIV positive*				*HIV negative*			
	*Used drugs**	*Did not use drugs*	*Drug use unknown*	*Total*	*Used drugs*	*Did not use drugs*	*Drug use unknown*	*Total*
Brooklyn Addictive Service	25	1	4	30	40	2	2	44
Haitian	0	16	1	17	0	39	0	39
Outside referrals	6	19	2	27	0	5	0	5
Einstein	8	7	0	15	13	21	1	35
Bronx Lebanon	1	1	0	2	1	2	0	3
TOTAL	40	44	7	91	54	69	3	126

*Cocaine, methadone, heroin, dolophine, or other narcotics.

inclusion (almost exclusively) of intravenous drug users.[8, 9] In addition, previous reports from developed nations have analyzed only 25 to 30 births to seropositive patients with a concomitant limitation in the statistical power of results.[8, 9] Finally, recent data on the effect of maternal serologic status on birth outcomes[10] have been in conflict with initially reported data.[8, 9]

To address these and other concerns, we undertook a prospective study of HIV-positive pregnant women (predominantly asymptomatic) and their offspring and comparable HIV-negative women from the same clinics. Although this cohort study is designed to provide information on the natural history of HIV disease in mothers and children for several years after an index pregnancy, this report is restricted to a comparison of the health status at birth of infected and uninfected mothers and their infants.

Methods

Population. From June 26, 1985, to Feb. 24, 1989, HIV-infected and uninfected prenatal patients at clinics in Brooklyn and the Bronx were recruited into a prospective cohort study. Except at the Kings County Hospital Center's clinic for prenatal Haitian women (see below), all women who agreed to be tested for HIV and who agreed to remain in follow-up through pregnancy and to allow subsequent follow-up of themselves and their infants were included in the study (Table I). Thirty of 77 patients at the Kings County Hospital Addictive Disease Clinic (Brooklyn Addictive Service in Table I) were seropositive. Referred patients in Brooklyn (outside referrals) who came for HIV testing and were found to be HIV positive, were recruited as were seronegative patients from the same source (20.6% of these patients are drug users). At Jacobi Hospital and Bronx Lebanon Hospital in the Bronx, prenatal patients with recognized risk for HIV infection (i.e., intravenous drug use or sexual partner of drug user) were referred by clinic staff to study personnel and offered HIV testing. All such referrals (seropositive and seronegative) were offered study entry. Because only about 6% of women at the Kings County Hospital Center's Prenatal Clinic for Haitian women were seropositive, only two seronegative women were selected for each seropositive woman at this clinic. The seronegative women who were selected to enter the study were in the same trimester of pregnancy as the seropositive women at entry. Unregistered patients were not evaluated in this study.

Data are available on 101 seropositive patients and 129 seronegative patients who consented to entry the study. An additional 11 seropositive women refused entry or never returned for results. All participants completed their pregnancies or, in the case of abortions, have passed their estimated dates of confinement. Among seronegative patients, 124 (96%) had live births, 2 (2%) had stillbirths, and 3 (2%) underwent intentional abortion. Among seropositive patients, 91 (90%) had live births, one had a spontaneous abortion, and 9 (9%) had intentional abortions. This study focuses on the 91 HIV-seropositive and 126 HIV-seronegative mothers who gave birth and for whom outcome data are available. Twins born to two of the HIV-seropositive mothers and stillbirths born to two seronegative mothers were excluded from infant evaluations. The mothers of twins were also excluded from analyses of obstetric complications.

Outcome measures. Obstetric outcomes were defined as follows: premature rupture of membranes, rupture of membranes before the onset of labor (since women undergoing electively scheduled cesarean section were at less risk for premature rupture of membranes, they were excluded from this analysis); maternal fever, two temperature readings of $\geq 101°$ F during the intrapartum or postpartum period; chorioamnionitis, women in labor with a temperature $>100.4°$ F without an extrauterine cause. Because some patients were uncertain about their last menstrual period and

some patients did not have early ultrasonographic evaluation, gestational age was based on physical examination at birth. In addition, standard physical measurements and Apgar scores were recorded at birth.

T-cell analyses. Lymphocyte phenotyping on a flow cytometer (Epics C, Coulter Electronics, Inc., Hialeah, Fla.) was performed with directly fluoresceinated monoclonal antibody reagents OKT4 and OKT8 (Ortho Diagnostics, Raritan, N.J.) as phenotypic markers of $T4^+$ and $T8^+$ T-lymphocyte subsets, respectively. Absolute T-cell counts were the product of the proportion of cells staining with each marker and the total lymphocyte count as measured by complete blood count and differential.

Statistical methods. Quantitative variables were compared with a two-sample t test, and proportions were compared with a χ^2 test unless any cell count was <5; then two-sided Fisher's exact test was used. Significant levels were adjusted using the procedure of Mantel and Haenszel[11] for stratified analysis of contingency tables. Adjusted estimates of HIV effects on birth weight and other infant birth characteristics were obtained from multivariate linear regressions that included HIV status (1 if seropositive, 0 otherwise) and additional covariates that affect fetal growth. The symbol NS indicates that a difference is not statistically significant ($p > 0.05$).

Results

Women. Infectious, obstetric, and medical complications are detailed in Table II. Seropositive patients had significantly more (17.6%) sexually transmitted diseases than seronegative patients (7.1%). Chorioamnionitis, endometritis, and maternal fever occurred among 2 (2.4%), 4 (4.4%), and 12 (14.3%) of the seropositive women and 6 (5.0%), 3 (2.4%), and 11 (9.2%) of the seronegative women, respectively (NS). None of these differences was statistically significant in a stratified analysis to control for delivery by cesarean section (the cesarean section rates for seropositive and seronegative patients were 12% and 18%, respectively). Other infectious complications (e.g., urinary tract infections, respiratory infections) occurred in 9 (9.9%) seropositive women and 5 (4.0%) seronegative women (NS). There were three opportunistic infections included among the seropositive women's infections (described in detail in a previous publication[12]).

Premature rupture of membranes occurred in 21 of 109 (19.3%) seronegative patients and 25 of 80 (31.2%) seropositive patients (NS); this analysis excludes patients who had elective cesarean sections. No additional obstetric complications, including placental problems, malpresentations, toxemia, or hemorrhage, were associated with serologic status.

Medical complications were noted in 37 (43.0%) seropositive and 30 (25.0%) seronegative woman ($p = 0.006$). Although the total number of complications was greater among seropositive patients, no specific type of complication (e.g., hypertension or diabetes) was significantly associated with serologic status.

Infants. Birth outcomes are shown in Table III. The mean gestational age (± SEM) and birth weight of children of seronegative mothers are 38.8 ± 2.3 weeks and 3014 ± 676 gm compared with 38.2 ± 2.6 weeks and 2906 ± 657 gm for children of seropositive mothers (NS). There was no significant difference when all infants or only those whose mothers had the same drug use status were compared. Similarly, weight/age ratios, heights, and head circumferences did not differ significantly. The mean 1- and 5-minute Apgar scores of children of seronegative mothers were 7.7 ± 1.7 and 8.7 ± 1.1, whereas for children of seropositive mothers the scores were 7.6 ± 1.9 and 8.7 ± 1.2 (NS).

Multivariate linear regression models were used to adjust all analyses in Table IV for site of clinic and mother's prenatal drug status. In addition, mother's cigarette use and age at delivery were inversely associated with birth weight, height, and head circumference and were included in the regression analyses of those outcomes. The last column indicates which covariates were included in the regression models, in addition to HIV serologic status (coded 1 or 0); the coefficients provide an indication of the degree to which the covariates influence the response and in what direction. For example, the number −389 in the birth weight row indicates that mothers who smoke >20 cigarettes per day have infants weighing 389 gm less than infants of nonsmokers. The purpose of this multivariate regression was not primarily to estimate the impact of covariates such as smoking, but to yield a valid comparison between HIV-positive and HIV-negative mothers, even if important characteristics, such as heavy smoking, tended to be more prevalent in one group of mothers than the other. Other covariates (e.g., alcohol use) were considered for inclusion in these models but were not used because they were not statistically significantly associated with birth outcomes in models containing clinic and drug use status.

Table IV shows that maternal HIV status was not significantly associated with size, birth weight, head circumference, or Apgar score in these analyses. The adjusted effect of positive maternal HIV serologic status (± 1.96 SE) was 7.15 ± 187 gm on birth weight, −0.045 ± 1.10 cm on height, and −0.085 + 0.684 cm on head circumference. Additional models adjusting for the effect of maternal T_4-cell level (measured within 2 months of birth) in addition to the factors cited above

Table II. Maternal complications during pregnancy

	HIV negative	*HIV positive*	*Unadjusted significance*	*Adjusted significance**
Infectious complications				
Sexually transmitted diseases†	9/126 (7.1%)	16/91 (17.6%)	$p = 0.017$	$p = 0.018$
Drug use	5/54 (9.3%)	10/40 (25.0%)	$p = 0.039$	
No drug use	4/69 (5.8%)	5/44 (11.4%)	NS	
Maternal fever‡	11/119 (9.2%)	12/84 (14.3%)	NS	NS
Drug use	4/50 (8.0%)	6/39 (15.4%)	NS	
No drug use	7.67 (10.4%)	5/39 (12.8%)	NS	
Chorioamnionitis‡	6/119 (5.0%)	2/84 (2.4%)	NS	
Endometritis‡	3/126 (2.4%)	4/91 (4.4%)	NS	
Other infection§	5/126 (4.0%)	9/91 (9.9%)	NS	
Obstetric complications				
Abnormal presentation	7/118 (5.9%)	4/84 (4.7%)	NS	
Placenta previa	2/118 (1.7%)	1/85 (1.2%)	NS	
Abruptio placentae	3/118 (2.5%)	0/85 (0.0%)	NS	
Retained placenta	1/126 (1.0%)	3/89 (3.4%)	NS	
Toxemia	4/118 (3.4%)	1/85 (1.2%)	NS	
Premature labor	9/78 (11.5%)	13/56 (23.2%)	NS	
Premature rupture of membranes (excludes elective cesarean sections)	21/109 (19.3%)	25/80 (31.2%)	NS	NS
Drug use	8/45 (17.8%)	16/35 (45.7%)	$p = 0.007$	
No drug use	13/62 (21.0%)	7/39 (17.9%)	NS	
Peripartum hemorrhage	5/126 (4.0%)	4/89 (4.5%)	NS	
Medical complications				
Endocrine	6/126 (4.8%)	5/91 (5.5%)	NS	
Cardiovascular	6/126 (4.8%)	6/91 (6.6%)	NS	
Any medical	30/120 (25.0%)	37/86 (43.0%)	$p = 0.006$	$p = 0.007$
Drug use	16/50 (32.0%)	16/39 (41.0%)	NS	
No drug use	12/67 (17.9%)	15/41 (36.6%)	$p = 0.030$	

*Significance levels were adjusted by stratification on drug use with the procedure of Mantel and Haenszel.

†Sexually transmitted diseases include syphilis, gonorrhea, hepatitis, herpes, condyloma, and genital ulcers diagnosed during pregnancy.

‡No difference noted when each mode of delivery was studied individually (i.e., vaginal, elective cesarean, nonelective cesarean).

§Other infections include respiratory tract, urinary tract, *Candida*, etc.

did not reveal any significant associations between serologic status and birth outcomes. Regression models restricted to only those infants with birth weights <2500 gm and to infants born at <37 weeks' gestation were also performed and revealed no significant associations with serologic status.

To assess the effect of more advanced immunodeficiency on outcomes, we separately assessed women whose last T_4-cell level before delivery was <300/mm³. There were only five such women, however, and their outcomes did not vary significantly from those of other seropositive women.

Comment

Our data indicate that maternal serologic status in a population of women with low acquired immunodeficiency syndrome (AIDS) prevalence (approximately 3% of the seropositive women had AIDS) has little or no demonstrable impact on the course of pregnancy or on the size, weight, and Apgar scores of live neonates. These findings are in agreement with those of Selwyn et al.,[9] who studied American women who used drugs, and with those of Johnston et al.[8] who studied women drug users in Edinburgh. Our findings extend those of previous studies by including seropositive women without a history of drug abuse. However, it should be mentioned that the power of the study to detect adverse effects of HIV infection on infrequent complications of pregnancy, such as endometritis or toxemia (Table II) is limited, and additional data on such infrequent complications would be useful.

These results differ from the report of Ryder et al.[10] who found that children born to seronegative women in Zaire weighed 3107 gm, on average, compared with 2888 gm for children of seropositive women. However, most of this difference of 219 gm is attributable to the 85 women with AIDS (18.2% of seropositives) whose children weighed only 2698 gm on average. In contrast, only 3% of the seropositive women in our study had AIDS at the time of delivery. Possibly, as advanced stages of immunodeficiency and AIDS develop in more women in the United States, the effects of seropositivity

Table III. Child's quantitative characteristics at birth, stratified by mother's prepartum drug use

	Mother HIV negative at birth			*Mother HIV positive at birth*			*Significance (two-tailed t test)*
	N	*Mean*	*SEM*	*N*	*Mean*	*SEM*	
Gestational age (wk) (by pediatrician)	115	38.8	2.3	81	38.2	2.6	NS
Drug use	49	37.9	2.6	37	37.6	2.8	NS
No drug use	63	39.4	1.7	40	38.9	2.3	NS
Size*	118	1.9	0.45	84	2.0	0.41	NS
Drug use	50	1.9	0.40	38	1.9	0.49	NS
No drug use	66	2.1	0.47	41	2.1	0.34	NS
Weight (gm)	120	3014	676	85	2906	657	NS
Drug use	51	2796	609	38	2680	629	NS
No drug use	67	3184	688	41	3147	632	NS
Weight/age ratio†	110	0.16	0.17	74	0.16	0.10	NS
Drug use	47	0.15	0.23	33	0.13	0.12	NS
No drug use	61	0.17	0.12	38	0.18	0.08	NS
Height (cm)	107	48.5	3.6	77	48.2	3.7	NS
Drug use	46	47.5	3.3	35	46.9	3.8	NS
No drug use	59	49.4	3.6	39	49.5	3.3	NS
Head circumference (cm)	108	33.5	3.1	78	33.2	2.4	NS
Drug use	46	32.6	1.6	35	32.5	2.6	NS
No drug use	60	34.2	3.8	40	33.9	2.1	NS
Total Apgar score (1 min)	113	7.7	1.7	82	7.6	1.9	NS
Drug use	47	7.6	1.9	38	7.5	2.1	NS
No drug use	64	7.7	1.5	39	7.6	1.7	NS
Total Apgar score (5 min)	113	8.7	1.1	82	8.7	1.2	NS
Drug use	47	8.7	1.3	38	8.7	1.4	NS
No drug use	64	8.8	0.9	39	8.7	1.2	NS

*Size is coded as follows: 1 = small for gestational age; 2 = at gestational age; 3 = large for gestational age.

†This ratio is calculated from Fig. 7-1 in Phibbs RH. The newborn infant. In: Rudolph AM, Hoffman, JIE. eds. Pediatrics. Norwalk, Connecticut: Appleton & Lange, 1987. The ratio is (weight − 2.289)/(age − 34) where weight is in kilograms and age is in weeks of gestation.

seen in Africa will become apparent. There are at least two mechanisms by which advanced maternal immunodeficiency could lead to low birth weight. First, the mother's health may suffer and she may be unable to provide an optimal environment for fetal growth. Second, immunodeficiency in the mother might promote direct viral infection in the fetus.[10] The differing results found in Africa may also reflect other aspects of the health of women there, including variations in virulence of HIV strains, social factors, and other causes of low birth weight, such as use of tobacco and drugs. All patients in our study had "wanted" pregnancies (i.e., they chose not to abort). Another possible contributor to the African findings is the fact that 71% of cases were from a hospital serving a lower socioeconomic clientele, whereas only 56% of controls were from that hospital.

Although our data give no indication that seropositivity is associated with birth outcome, the confidence intervals are broad enough to include some deficits (Table IV). For example, the 95% confidence interval for birth weight is −180 to 194. Thus the data do not rule out the possibility that seropositivity induces a deficit of 180 gm. However, larger deficits are not consistent with our data.

As expected, we found that birth weights were related to drug use and smoking as well as maternal age. After including these factors in regression models, as well as T_4-cell counts, and clinic setting, we found no significant effect of serologic status on birth weight or gestational age. The lack of a significant effect of positive serologic status on weight, height, or gestational age may not be generalizable to populations of women with more advanced immunodeficiency, as discussed previously.

We did note a trend toward more frequent infectious complications among seropositive women. Sexually transmitted diseases were diagnosed almost twice as often among seropositive women. This could reflect a life-style that predisposes to both HIV infection and the acquisition of other sexually transmitted diseases, an enhanced susceptibility to HIV infections among patients with sexually transmitted diseases, or a more protracted course of sexually transmitted diseases among HIV-infected women, leading to an increased likelihood of diagnosis of sexually transmitted diseases

Table IV. Regression models to adjust the effect of maternal HIV status on child's quantitative outcomes for other factors

Dependent variable	*n*	*Adjusted effect of positive maternal HIV status (with 95% confidence interval)*		*SE*	*Significance*	*Other independent variables in the model (with regression coefficients)**
Size	191	0.034	(−0.10-0.17)	0.070	NS	Outside (0.087), Haitian (0.184), Einstein (0.020), drug use (−0.073)
Birth weight (gm)	182	7.15	(−180-194)	95.5	NS	Outside (40.8), Haitian (302), Einstein (126), drug use (−23.2), mother's age at delivery (−25), smoked <20 (−250), smoked ≥20 (−389)
Birth weight (cm)	167	−0.045	(−1.14-1.06)	0.561	NS	Outside (0.331), Haitian (1.176), Einstein (−1.291), drug use (−1.06), mother's age at delivery (−0.105), smoked <20 (−0.300), smoked ≥20 (−1.001)
Birth head circumference (cm)	170	−0.085	(−0.77-0.60)	0.349	NS	Outside (0.129), Haitian (0.702), Einstein (0.102), drug use (−0,587), mother's age at delivery (−0.063), smoked <20 (−0.911), smoked ≥20 (−0.979)
Apgar score (1 min)	185	−0.015	(−0.60-0.56)	0.296	NS	Outside (0.282), Haitian (0.805), Einstein (0.671), drug use (0.330)
Apgar score (5 min)	185	−0.006	(−0.39-0.38)	0.197	NS	Outside (0.282), Haitian (0.480), Einstein (0.456), drug use (0.181)

*All analyses were adjusted for clinic and mother's prenatal drug use status. Mother's smoking status and age at delivery were associated with birth weight, height, and head circumference and were included in the analysis of these outcomes. The four patients from Bronx Clinic were not included. Dummy variables coded 1.0 or 0.0 indicated referral from the outside, Haitian, or Einstein clinics, whether the mother used drugs, and whether the mother smoked 1 to 19 cigarettes per day or at least 20 cigarettes per day during the first trimester. Mother's age at delivery was coded in years. Size is coded as in Table III. Additional models for the mother's last known thyroxine level, as well as the above factors, were performed but did not result in any significant associations with birth outcomes.

at any given point in time. The association of HIV with sexually transmitted diseases in pregnancy has clinical consequence because many of these infections, particularly syphilis and chlamydia, can lead to perinatal morbidity. Therefore assiduous screening for sexually transmitted diseases at first contact and again in the third trimester seems prudent. Since the efficacy of standard therapies for sexually transmitted diseases in seropositive pregnant women remains largely unstudied, it would seem appropriate to obtain tests of cure. The trend toward a higher incidence of premature rupture of membranes among seropositive women could also reflect a change in vaginal flora that has been linked to premature rupture of membranes.[13]

As expected, opportunistic infections were seen only in the seropositive cohort. A previous report from an overlapping cohort has shown that, as in nonpregnant patients, low T_4-cell counts are predictive of risk for infections among seropositive pregnant women.[12] That report noted a substantial risk of serious infections during pregnancy among those whose T_4-cell counts decreased to $<300/mm^3$. However, opportunistic infections were uncommon among our seropositive patients. Although rare, these serious infections carry potentially devastating consequences for mother and child. However, with the newer treatment modalities, such as aerosolized pentamidine, it may be possible to postpone clinical illness beyond pregnancy. Thus it would be useful to find markers to identify the subset of patients at highest risk for opportunistic infections.

The pregnancy outcomes in these women were more influenced by drug use and smoking than by detectable viral effects. No clinically significant differences in birth weight, birth height, head circumference, or Apgar scores were related to maternal serologic status. Therefore it seems that in populations of relatively healthy seropositive pregnant women both those offspring who will eventually become infected with HIV and those who will not become infected can benefit from the application of conventional precepts of prenatal care. In particular, every effort should be made to encourage the prospective mother to stop using drugs, cigarettes, and alcohol and to reduce exposures to sexually transmitted diseases. In addition, obstetricians should direct increased attention to screening for sexually transmitted diseases, to treatment of these infections, and to

careful posttreatment assessment of the mother's infection status.

REFERENCES

1. Novick LF, Berns D, Stricof R, Stevens R, Ross K, Wethers J. HIV seroprevalence in newborns in New York state. JAMA 1989;261:1745-50.
2. Hoff R, Virardi VP, Weiblen BJ, Mahoney-Trout L, Mitchell ML, Grady GF. Seroprevalence of human immunodeficiency virus among childbearing women: estimating by testing samples of blood from newborns. N Engl J Med 1988;318:525-30.
3. Landesman S, Minkoff H, Holman S, McCalla S, Sinjin O. Serosurvey of human immunodeficiency virus infection in parturients. JAMA 1987;258:2701-3.
4. Centers for Disease Control. Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotrophic virus type III/lymphadenopathy associated virus and acquired immunodeficiency syndrome. MMWR 1983;34:721-31.
5. American College of Obstetricians and Gynecologists. Prevention of human immune deficiency virus infection and acquired immune deficiency syndrome. Washington, DC: American College of Obstetricians and Gynecologists, 1987:1-4.
6. Lindsay MK, Peterson HB, Feng TI, Slade BA, Wallis S, Klein L. Routine antepartum human immunodeficiency virus infection screening in an inner city population. Obstet Gynecol 1989;74:289-94.
7. Minkoff HL, Landesman SH, Delke I, et al. Routinely offered prenatal HIV testing. N Engl J Med 1988; 319:1018.
8. Johnston FD, MacCallum L, Brethle R, Inglis JM, Peutherer JF. Does infection with HIV affect the outcome of pregnancy? Br Med J 1983;196:467.
9. Selwyn PA, Schoenbaum EE, Davenny K, et al. Prospective study of human immunodeficiency virus infection and pregnancy outcomes in intravenous drug users. JAMA 1989;261:1289-94.
10. Ryder RW, Nsa W, Hassia SE, et al. Prenatal transmission of the human immunodeficiency virus type 1 to infants of seropositive women in Zaire. N Engl J Med 1989; 320:1837-42.
11. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of diseases. JNCI 1959;22:719-48.
12. Minkoff HL, Willoughby A, Mendez H, et al. Infectious morbidity during pregnancy among human immunodeficiency virus–infected women with severe immunocompromise. Am J Obstet Gynecol 1990;162:30-4.
13. Minkoff HL. Prematurity: is infection an etiology? Obstet Gynecol 1983;62:137-48.

Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femur length ratio

Gerald P. Marquette, MD,[a] Marc Boucher, MD,[a] Micheline Desrochers, RN,[b] and Louis Dallaire, MD[b]

Montreal, Quebec, Canada

Ultrasonographic determination of biparietal diameter/femur length ratios performed at 16 or 17 weeks' gestation in fetuses with trisomy 21 were not statistically different from those of 155 normal fetuses. It appears that this test could not be used for screening for trisomy 21. (Am J Obstet Gynecol 1990;163:1604-5.)

Key words: Ultrasonography, trisomy 21

Lockwood et al.[1] have suggested that Down syndrome may be associated with shortened femur lengths and elevated biparietal diameter/femur length ratios. These features would allow for a noninvasive screening technique applicable to a larger obstetric population than that of patients 35 years old or older. Recently we have had many consultations for ultrasonography on the basis of these skeletal features requesting use of the ratio to rule out trisomy 21 in women not considered at increased risk by maternal age. The concern about offering amniocentesis to such patients for fetal karyotyping motivated this study.

From the Departments of Obstetrics and Gynecology[a] and Medical Genetics,[b] Hôpital Sainte-Justine, University of Montreal.
Received for publication January 22, 1990; revised June 20, 1990; accepted June 25, 1990.
Reprint requests: Gerald P. Marquette, MD, Hôpital Sainte-Justine, Department of Obstetrics and Gynecology, 3175 Cote Sainte-Catherine Road, Montreal, Quebec, Canada H3T-1C5.
6/1/23412

Methods

This cohort study comprised 31 fetuses with trisomy 21 and 155 control fetuses without anomalies. The subjects were drawn from our antenatal genetic diagnostic clinic population. All pregnancies were referred for advanced maternal age ≥35 years. All ultrasonographic and amniocentesis techniques were performed at 16 or 17 weeks by the same team over a period of 3½ years. All ultrasonographic data were gathered with a Siemens Sonoline SL-2 or AC unit with a linear array 3.5 MHz transducer. The biparietal diameter was measured from a leading edge to a leading edge in a transaxial plane of the widest portion of the skull with the thalamus positioned in the midline. The femur was measured along the long axis of the diaphysis, disregarding the curvature. Control subjects were defined as the next five pregnancies after the diagnosis of trisomy 21 in a fetus with the same maternal age and parity, with no chromosomal or anatomic anomaly. As reported by Lockwood et al.,[1] we used a threshold of abnormality for the biparietal diameter/femur length ratio >1.5 SDs above the mean. A 2 × 2 contingency table was constructed for χ^2 analysis with Yates' correction.

Results

There was no significant difference for maternal age, parity, and gestational age at amniocentesis between both groups. The population studied was 92% white and was comparable in both groups. There were 6% blacks and 2% other races. Table I depicts the results in a 2 × 2 table of biparietal diameter/femur length ratio. There was no correlation between the diagnosis of trisomy 21 and an abnormal biparietal diameter/femur length ratio ($\chi^2 = 0.052$, $p = 0.82$).

Table I. Biparietal diameter/femur length ratios in fetuses with trisomy 21 and normal fetuses

Biparietal diameter/femur length	*Trisomy 21*	*Normal*
>1.5 SD	3	14
≤1.5 SD	28	141

$\chi^2 = 0.052$; $p = 0.82$.

Comment

Lockwood et al.[1] demonstrated a sensitivity of 70% with a threshold of 1.5 standard deviation above the mean for biparietal diameter/femur length ratio in a Boston population. In a New Haven population described in the same report the sensitivity was 51%. These authors further added that the positive predictive value of this test was greater than that of screening that is based on maternal age. Our report is a cohort study with matched controls, thus only the sensitivity can be reported; it was 10%. This figure varies greatly from 51% and 70%. Our population probably differs from both Boston and New Haven, but the biparietal diameter and femur length measurements in our study appear similar to previously published tables and cannot explain our different findings.

We conclude that the biparietal diameter/femur length ratio is not a suitable method for screening for trisomy 21 in our population. A recent report[2] on low maternal serum α-fetoprotein values as a screen for trisomy 21 seems more promising but remains to be confirmed in further studies.

REFERENCES

1. Lockwood C, Benacerraf B, Krinski A, et al. A sonographic screening method for Down Syndrome. AM J OBSTET GYNECOL 1987;157:803-8.
2. New England Regional Genetics Group Prenatal Collaborative Study for Down Syndrome Screening. Combining maternal α-fetoprotein measurements and age to screen for Down syndrome in pregnant women under age 35. AM J OBSTET GYNECOL 1989;160:575-81.

Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: An accurate and rapid method for detection of fetal chromosome abnormalities

Lee P. Shulman, MD,[a] Avirachan T. Tharapel, PhD,[b] Carole M. Meyers, MD,[a] Owen P. Phillips, MD,[a] Joe Leigh Simpson, MD,[a] and Sherman Elias, MD[a]
Memphis, Tennessee

Transabdominal chorionic villus sampling can be readily used for detection of fetal chromosome abnormalities in the second and third trimesters of pregnancy. Although culture of chorionic villi offers little advantage over cultured amniotic fluid cells with respect to time required to obtain results, cytogenetic analysis of chorionic villi by direct analysis of uncultured cytotrophoblastic cells offers clear advantages because of the very short time required to obtain results. To determine whether direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas can routinely provide rapid and accurate assessment of fetal status, we evaluated chorionic villus specimens obtained from 57 placentas; 49 placentas were sampled in the second trimester whereas eight were sampled in the third trimester. Direct preparations yielded karyotypes in 56 (98.2%) preparations; all results of direct analyses were available within 72 hours and, when requested, within 12 hours. All results were confirmed by chromosome analysis of cultured mesenchymal core cells or cultured fetal tissue. We conclude that direct analysis of cytotrophoblastic cells from second- and third-trimester placentas is a very rapid and accurate method for determining fetal chromosome status that is comparable with, if not superior to, percutaneous umbilical blood sampling. (Am J Obstet Gynecol 1990;163:1606-9.)

Key words: Prenatal diagnosis, chorionic villi, cytogenetics, second and third trimester

Transabdominal chorionic villus sampling can be used to detect fetal chromosome abnormalities in the second and third trimesters of pregnancy.[1-7] Although culture of chorionic villi (mesenchymal core) offers relatively little advantage over cultured amniotic fluid cells with respect to time required to obtain results, cytogenetic analysis of chorionic villi is also possible by the so-called direct analysis of uncultured cytotrophoblastic cells. Direct analysis of uncultured cytotrophoblastic cells provides a rapid assessment of fetal chromosome complement in the first trimester,[8,9] with results available within hours if needed. This interval is potentially even shorter than the 48 to 72 hours required to obtain cytogenetic results from fetal blood samples obtained by percutaneous umbilical blood sampling[10] because direct analysis can be obtained the same day if necessary. We reasoned that direct cytogenetic analysis of cytotrophoblastic cells could offer substantial advantage over other methods used for second- and third-trimester prenatal diagnosis (e.g., cultured amniotic fluid cells, cultured lymphocytes obtained by fetal blood sampling). This article reports our cytogenetic experience with 57 chorionic villus samples, obtained by either transabdominal chorionic villus sampling or pregnancy termination during the second and third trimesters of pregnancy.

From the Departments of Obstetrics and Gynecology[a] and Pediatrics,[b] the University of Tennessee, Memphis.
Supported in part by the National Institutes of Health (grant numbers HD-82904, HD-22968), March of Dimes, Agency for International Development, and a University of Tennessee Chair of Excellence (J.L.S.)
Presented at the Tenth Annual Meeting of the Society of Perinatal Obstetricians, Houston, Texas, January 23-27, 1990.
Received for publication May 1, 1990; revised July 17, 1990; accepted July 20, 1990.
Reprint requests: Lee P. Shulman, MD, Department of Obstetrics and Gynecology, University of Tennessee, Memphis, 853 Jefferson Ave., Room E-102, Memphis, TN 38163.
6/1/24033

Material and methods

Chorionic villus specimens were obtained during a 22-month period (1988 to 1989) from patients undergoing either transabdominal chorionic villus sampling during the second or third trimester (≥14 weeks' gestation) or second trimester (14 to 24 weeks' gestation) pregnancy termination. Gestational age was determined by the patient's last menstrual period and was confirmed by ultrasonographically derived biometric measurements. The entire villus specimen was used if chorionic villi were obtained by transabdominal chorionic villus sampling; 15 to 20 mg of chorionic villi was obtained for cytogenetic analysis from second-trimester pregnancy termination placental specimens; this amount was comparable with amounts we

routinely obtain by transabdominal chorionic villus sampling.

Villi were cleaned and incubated in complete medium (α-minimum essential medium supplemented with 15% Chang medium, 15% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin) at 37° C for 3 to 15 hours. The incubation time period was determined on the basis of need for rapid (<12 hours) cytogenetic results and the time of day the sample was received in the laboratory. All chorionic villi were treated simultaneously with trypsin–ethylenediaminetetraacetic acid to separate cytotrophoblastic cells from mesenchymal core and with Colcemid to accumulate metaphases.[11] Cytotrophoblastic cells were detached from chorionic villi by vigorously agitating villi with a Pasteur pipette, resuspension in complete medium, and centrifugation at 1000 rpm for 6 minutes. The supernatant was discarded and the pellet was resuspended in 5 ml hypotonic (1 : 1 mixture of 0.075 mol/L potassium chloride and 0.8% sodium citrate) solution. Cells were subsequently fixed thrice with a 3 : 1 methanol:glacial acetic acid solution. Slides were then prepared and stained with Giemsa by conventional methods.

Remaining pieces of villi, which represent portions of mesenchymal core, were used to initiate culture analyses.[11] When fetal tissue (e.g., blood, skin) was available, culture analyses were initiated to corroborate direct cytogenetic results. Conventional tissue culture techniques were used to perform culture analyses of fetal tissues.

Results

Chorionic villus specimens were obtained from 57 subjects. Ten women had undergone transabdominal chorionic villus sampling for genetic diagnosis at or after 18 weeks' gestation (range, 18 to 37 weeks). Of those who had transabdominal chorionic villus sampling, eight women were in the third trimester. In all 10 transabdominal chorionic villus sampling cases, chorionic villus samples were obtained after a single ultrasonographically guided passage of an 18-gauge spinal needle. There were no complications (infection, hemorrhage, stillbirth) among these 10 women who had second- and third-trimester transabdominal chorionic villus sampling. Forty-seven subjects underwent second-trimester (range, 14 to 24 weeks) pregnancy termination for elective or genetic (e.g., fetal chromosome abnormalities, neural tube defects) indications.

Sufficient tissue (≥5 mg of chorionic villi) to initiate both direct and culture cytogenetic analyses was available from all 57 specimens. Transabdominal chorionic villus sampling yielded an average of 14.8 mg villi (±2.7 SD; range, 5 to 30 mg) for cytogenetic analysis. We sought to mimic this amount in obtaining tissue for cytogenetic analysis from second-trimester pregnancy terminations; an average of 17.8 mg villi (±1.3 SD; range 15 to 20 mg) was actually obtained from these specimens. Forty-nine patients were in the second trimester (14 to 24 weeks' gestation) and eight were in the third trimester (25 to 37 weeks) at the time of chorionic villi collection.

Direct analysis of cytotrophoblastic cells yielded metaphases suitable for cytogenetic analysis in 56 of 57 (98.2%) cases. At least eight metaphases were analyzed from each direct preparation. In all cases results were available within 3 days (mean, 1.8 days ± 0.53 SD; range, 0.5 to 3 days). In the only three cases in which results were requested in <24 hours, rapid diagnosis was achieved. Of the 56 cases in which metaphases were obtained, 48 (85.7%) revealed normal complements (46,XY or 46,XX) and eight (14.3%) revealed abnormal complements (three cases of trisomy 21 and one case each of trisomy 18, 45,X, 46,XY,−22,+der(9), 47,XXY, and 69,XXX, respectively). The sole direct failure occurred in a 5 mg chorionic villus specimen obtained by transabdominal chorionic villus sampling at 19 weeks and incubated for 15 hours; cytogenetic results (46,XY) were later obtained by analysis of cultured mesenchymal core cells.

For confirmation of results obtained by direct analysis, cultured mesenchymal core cells were used to obtain metaphases in the 57 cases. Results were available within 17 days (mean, 10.8 days ± 3.8 SD; range, 6 to 17 days). In addition, fetal tissue (fibroblasts) for cytogenetic analysis was obtained from all pregnancy termination cases and yielded suitable metaphases in all 47 cases. Results obtained from direct analyses were corroborated in all cases.

Comment

Cytogenetic analysis of chorionic villi from second- and third-trimester placentas is known to be a reliable method for obtaining fetal cytogenetic information.[1-7] Direct analysis of first-trimester chorionic villi (cytotrophoblastic cells) provides a more rapid method for obtaining cytogenetic results,[8, 9, 12] and offers potential clinical advantages in the second and third trimesters. Although so-called direct analysis of chorionic villi has been reported in second- and third-trimester chorionic villus specimens, much variation exists in the definition of "direct analysis." Not all techniques actually produce results as rapidly as their name implies. Results are sometimes not available until at least 3 to 4 days, an interval that offers no advantage over that in routine culture of lymphocytes obtained by percutaneous umbilical blood sampling. For example, Hogdall et al.[5] used a "48 hour short-term incubation period" before cell fixation. By contrast, at our institution we restrict the term "direct analysis" to cytogenetic evaluation of

separated cytotrophoblastic cells that are incubated for only a short (3 to 15 hours) time period before cell fixation. The mean time from procedure to preliminary results is 1.8 days, compared with 10.8 days for analyses of cultured villi. In this study, chorionic villus samples were obtained from 49 placentas ≤24 weeks' gestation and eight placentas >24 weeks' gestation; direct preparations from all third-trimester cases provided adequate metaphase spreads for cytogenetic analysis. Furthermore, in those three instances in which rapid diagnosis was required for diagnostic purposes, results were available within 24 hours. Our findings are thus comparable with those of Holzgreve et al.[3] and Basaran et al.[4] who followed the technique of Simoni et al.,[13] who used a 1.25-hour incubation period before cell fixation and usually obtained cytogenetic results within 48 hours.

Evaluation of fetal cytogenetic complement in the second and third trimester is not infrequently necessary, and sometimes direct analysis of uncultured cytotrophoblastic cells is the only method to provide critical information. In the second trimester cytogenetic analysis is useful for those patients who are seen so late in gestation that amniocentesis could not provide information with regard to fetal status in sufficient time to allow pregnancy termination. In the third trimester cytogenetic analysis is as applicable as percutaneous umbilical blood sampling for patients at increased risk for fetal chromosome abnormalities. Indications include nonlethal malformations detected by ultrasonography or unexplained intrauterine growth retardation. Transabdominal chorionic villus sampling is the only plausible alternative if percutaneous umbilical blood sampling is not technically possible (e.g., oligohydramnios). Moreover, conventional analysis of lymphocytes obtained from percutaneous umbilical blood sampling usually requires a longer time (48 to 72 hours) to obtain results than is required by direct analysis of cytotrophoblastic cells (12 hours, if necessary).[13] Of note, however, is our previous description of a technique in which spontaneously dividing cells in fetal blood can be used to obtain cytogenetic results within 24 hours.[10, 14]

Although initial results are promising, the safety and accuracy of second- and third-trimester transabdominal chorionic villus sampling remain to be assessed fully. Initial reports indicate that transabdominal chorionic villus sampling in the first and second trimesters is a relatively safe procedure[5-7]; however, the safety of transabdominal chorionic villus sampling in the second and third trimesters has yet to be addressed. Our own direct preparations were all confirmed by analyses of cultured mesenchymal core cells or cultured fetal tissue, but additional data are still necessary to fully evaluate the diagnostic accuracy of cytogenetic analysis of cytotrophoblastic cells obtained from second- and third-trimester placentas. Discrepancies between cytogenetic analysis of cytotrophoblastic cells and mesenchymal core cells obtained from first-trimester placentas are well documented,[8, 9, 12] and it would not be surprising if similar discrepancies existed later in pregnancy. Indeed reports from several centers[2, 15-17] indicate that diagnostic problems (e.g., placental mosaicism) encountered in cytogenetic analysis of chorionic villi from first-trimester placentas similarly have been encountered in cytogenetic analysis of second- and third-trimester chorionic villi.

REFERENCES

1. Nicolaides KH, Rodeck CH, Soothill PW, Warren RC. Why confine chorionic villus (placental) biopsy to the first trimester? Lancet 1986;1:543-4.
2. Hugentobler W, Binkert F, Haenel AF, Schaetti D. Die Chorionzotten-(Plazenta-) Biopsie im II. und III. Trimenon: Neue Perspektiven der Pranataldiagnostik. Geburtshilfe Frauenheilkd 1987;47:729-32.
3. Holzgreve W, Miny P, Basaran S, Fuhrmann W, Beller FK. Safety of placental biopsy in the second and third trimesters [Letter]. N Engl J Med 1987;317:1159.
4. Basaran S, Miny P, Pawlowitzki I-H, Horst J, Holzgreve W. Rapid karyotyping for prenatal diagnosis in the second and third trimesters of pregnancy. Prenat Diagn 1988; 8:315-20.
5. Hogdall CK, Doran TA, Shirne J, Wilson S, Teshima I. Transabdominal chorionic villus sampling in the second trimester. Am J Obstet Gynecol 1988;158:345-9.
6. Jahoda MG, Pijpers L, Reuss A, Sachs ES. Transabdominale Choriozentese fur die schnelle pranatale Diagnostik im zweiten Trimenon: Erfahrungen bei 147 Schwangeren. Z Geburtschilfe Perinatol 1988;192:101-3.
7. Pijpers L, Jahoda MG, Reuss A, Wladimiroff JW, Sachs ES. Transabdominal chorionic villus biopsy in second and third trimesters of pregnancy to determine fetal karyotype. Br Med J 1988;297:822-3.
8. Canadian Collaborative CVS-Amniocentesis Clinical Trial Group. Multicentre randomised clinical trial of chorion villus sampling and amniocentesis. First report. Lancet 1989;1:1-6.
9. Rhoads GG, Jackson LG, Schlesselman SE, et al. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. N Engl J Med 1989;320:609-17.
10. Tipton RE, Tharapel AT, Chang H-H, Simpson JL, Elias S. Rapid chromosome analysis with the use of spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal). Am J Obstet Gynecol 1989; 161:1546-8.
11. Tharapel AT, Elias S, Shulman LP, Seely L, Emerson DS, Simpson JL. Resorbed co-twin as an explanation for discrepant chorionic villus results: non-mosaic 47,XX,+16 in villi (direct and culture) with normal (46,XX) amniotic fluid and neonatal blood. Prenat Diagn 1989;9:467-72.
12. Ledbetter DH, Martin AO, Verlinsky Y, et al. Cytogenetic results of chorion villus sampling: high success rate and diagnostic accuracy in the U.S. Collaborative Study. Am J Obstet Gynecol 1990;162:495-501.
13. Simoni G, Gimelli G, Cuoco C, et al. First trimester fetal karyotyping: one thousand diagnoses. Hum Genet 1986; 72:203-9.

14. Tharapel AT, Moretti M, Meyers CM, et al. Diagnosis of trisomy 18 using spontaneously dividing cells from fetal umbilical cord blood: a novel approach for rapid late second and third trimester prenatal diagnosis. Am J Perinatol 1990;7:211-3.
15. Eiben B, Leipoldt M, Schubbe I, Ulbrich R, Hansmann I. Partial deletion of 4p in fetal cells not present in chorionic villi. Clin Genet 1988;33:49-52.
16. Leschot NJ, Wolf H, Weenick GH. False negative findings at third trimester chorionic villus sampling (C.V.S.). Clin Genet 1988;34:204-5.
17. Miny P, Basaran S, Pawlowitzki I-H, et al. Validity of cytogenetic analyses from trophoblast tissue throughout gestation. Am J Med Genet 1989;33:136-41.

Perinatal mortality in Victoria, Australia: Role of group B streptococcus

John R. Fliegner, MD,[a] and Suzanne M. Garland, MD[b]
Parkville and Carlton, Australia

Group B β-hemolytic streptococcus is the most common infective cause of neonatal morbidity and mortality. It is therefore surprising that no agreement exists for an approach to its prevention. There is also increasing evidence that occult infection may play an etiologic role in premature rupture of the membranes and preterm labor. In this report we review the role of group B β-hemolytic streptococcal sepsis as a cause of perinatal wastage in the state of Victoria, Australia during the period 1982 to 1987. Group B β-hemolytic streptococcus accounted for 1.7% of overall perinatal deaths, and for 30.3% (77 of 254) perinatal deaths directly attributable to infection. By comparison, over the same 6-year period, erythroblastosis accounted for 0.5% of perinatal wastage and there were only two deaths as a result of congenital syphilis. The true incidence of lethal group B β-hemolytic streptococcal infection is probably greater because of the absence of histologic and bacteriologic studies in many perinatal deaths. We believe that intrapartum chemoprophylaxis with penicillin of all group B β-hemolytic streptococcus-positive carrier mothers would significantly reduce neonatal morbidity and mortality from this cause. (Am J Obstet Gynecol 1990;163:1609-11.)

Key words: Group B β-hemolytic streptococcus, perinatal wastage

The group B streptococcus organism is the most common cause of severe prenatal infection in our institution. The clinical signs of early onset disease are nonspecific and occur in other conditions associated with prematurity. The most frequent manifestation includes sepsis with pneumonia, bacteremia without a focus, or with meningitis. In some infants septic shock with neutropenia dominates the clinical picture and in these cases infection can be extremely fulminating and has an associated high mortality. The pathogenesis of infection is vertical transmission from a mother with genital group B β-hemolytic streptococcus colonization, and acquisition to the infant occurs just before delivery (through ruptured or intact membranes) or during birth canal passage. Although a few women have chorioamnionitis, most are asymptomatic before delivery and fail to signal any risk of infant infection unless preterm delivery occurs. Hematogenous spread may be prevented or aborted by early antibiotic treatment intrapartum. Infants may be infected in utero; this may be manifested clinically as fetal distress, late decelerations on fetal monitoring, and low Apgar scores at birth, or intrauterine death may occur. We report the impact of group B streptococcal infection on perinatal deaths for the State of Victoria, Australia, for the 6-year period from 1982 to 1987.

From the Department of Obstetrics and Gynaecology,[a] University of Melbourne, and the Microbiology Department,[b] Royal Women's Hospital.
Received for publication November 13, 1989; revised April 23, 1990; accepted June 15, 1990.
Reprint requests: John R. Fliegner, MD, Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, 3052, Victoria, Australia.
6/1/23235

Material and methods

One of the authors (J. F.) has been a member of the Consultative Council on Obstetric and Perinatal Mortality and Morbidity since 1973. Since 1962 this committee has reviewed all perinatal deaths in the state of Victoria on an annual basis. Victoria has a population of four million and an annual confinement rate of over

Table I. Perinatal mortality and role of infection as a cause in Victoria between 1982 and 1987

Year	*Total births (live births and still births)*	*Perinatal deaths (SB and NNDs)*	*Perinatal deaths caused by infections*	*Perinatal deaths caused by GBS infection*
1982	60,455	897	39 (4.4%)	10
1983	60,591	774	37 (4.8%)	7
1984	60,704	765	55 (7.2%)	20
1985	61,176	741	37 (5.0%)	10
1986	61,253	680	48 (7.1%)	17
1987	61,474	677	38 (5.6%)	13
TOTAL	365,653	4534	254 (5.6%)	77 (1.7%)

SB, Stillbirth; *NND*, neonatal death; *GBS*, group β streptococcal.

60,000. The perinatal mortality and rate of infection for the study period were reviewed. Specific attention was paid to the role of group B β-hemolytic streptococcus sepsis as a cause of perinatal wastage.

Definitions of stillbirth and neonatal death as defined by the Victorian Council are as follows: stillbirth—a stillborn infant weighing ≥500 gm, or, if the weight is not known, born after at least 22 weeks' gestation; neonatal death—a death occurring within 28 days of birth in an infant whose birth weight was ≥500 gm or, if the weight is not known, an infant born after at least 22 weeks' gestation; perinatal mortality rate—the number of perinatal deaths (stillbirths plus neonatal deaths) per 1000 births (live births and stillbirths).

Results

The overall perinatal mortality, the role of infection, and perinatal deaths caused by group B β-hemolytic streptococcus are summarized in Table I. For the 6-year period (1982 to 1987) the occurrence of infection as the direct cause of perinatal death fluctuated between 4.4% and 7.2%. However, group B β-hemolytic streptococcus infections for the entire period have been the most common causative agent responsible. In 1984 there were 20 losses attributed to group B β-hemolytic streptococcus infection; this accounted for 2.5% of all perinatal deaths and 36.4% of all infection cases. By comparison, during this 6-year period there were 24 perinatal deaths as a direct result of *Escherichia coli* infection, and only two from syphilitic infection. During the same period erythroblastosis accounted for 23 (0.5%) of total perinatal deaths.

In our institution (Royal Women's Hospital, Carlton) during the 8-year period from 1981 to 1988 a total of 30,197 live births were analyzed in which prenatal group B β-hemolytic streptococcus screening at 32 weeks was performed. There were no cases of neonatal group B β-hemolytic streptococcus sepsis among infants of asymptomatic carrier women who received intrapartum penicillin prophylaxis. By contrast, there were 27 infections with nine deaths in a control group of 26,915 infants when maternal group B β-hemolytic streptococcus screening had not occurred.

Comment

We analyzed the importance of group B β-hemolytic streptococcus disease as a cause of perinatal wastage in Victoria, Australia, during the 6 years from 1982 to 1987. During this period group B β-hemolytic streptococcus infection was responsible for 77 of 254 perinatal deaths (30.3%) attributable to infection, and accounted for 1.7% of overall perinatal deaths.

There is increasing evidence that asymptomatic group B β-hemolytic streptococcus carriage may be followed by occult chorioamnionitis, premature rupture of membranes, and perinatal infection.[1] However, although the impact of early-onset group B β-hemolytic streptococcus sepsis on neonatal mortality and morbidity is well recognized, its role as a cause of unexplained stillbirth in late pregnancy is not. The pathologist may overlook the condition in the absence of inflammatory changes, or perinatal risk factors such as pyrexia, premature rupture of membranes, or premature labor. The diagnosis of fulminating group B β-hemolytic streptococcus sepsis is further complicated by the occurrence of maceration, delays in autopsy, and secondary bacterial contamination. It has been established that a major risk factor associated with the development of perinatal group B β-hemolytic streptococcus sepsis is the intensity of colonization of the lower genital tract at the onset of labor.[2] When maternal group B β-hemolytic streptococcus carriage rates are low, it is claimed that mass screening in pregnancy is unlikely to be cost effective.[3] Thus in the United Kingdom, with an overall rate of group B β-hemolytic streptococcus infections of 0.3 per 1000 live births, this would involve screening over 3000 women to detect one case of group B β-hemolytic streptococcus sepsis. The situation is different in the United States and Australia. In a recent U.S. report, the incidence of severe neonatal sepsis

caused by group B β-hemolytic streptococcus infection was 4.62 cases per 1000 births, with a neonatal mortality of 0.8 per 1000 live births.[4]

In conclusion, we think that potentially avoidable perinatal deaths (in addition to serious neonatal sepsis) will continue unless the question of maternal group B β-hemolytic streptococcus colonization is addressed. At the Royal Women's Hospital, Melbourne, Australia, we have established that intrapartum penicillin prophylaxis in women with positive group B β-hemolytic streptococcus cultures at 32 weeks' gestation can prevent early-onset group B β-hemolytic streptococcus sepsis. Such a procedure is simple, cost effective, and may have important medicolegal implications in the future.

We thank the Consultative Council on Obstetric and Paediatric Mortality and Morbidity in Victoria for permission to use data from the annual reports covering the years 1982 to 1987.

REFERENCES

1. Galask RP, Varner MW, Petzold R, Wilbur SL. Bacterial attachment to the chorioamniotic membranes. AM J OBSTET GYNECOL 1984;148:915-28.
2. Tuppurainen N, Hallman M. Prevention of neonatal group B streptococcal disease: intrapartum detection and chemoprophylaxis of heavily colonized parturients. Obstet Gynecol 1989;73:583-8.
3. Easmon CSF, Hastings MJ, Neill J, Bloxham B, Rivers RPA. Is group B streptococcal screening during pregnancy justified? Br J Obstet Gynaecol 1985;92:197-201.
4. Brady K, Duff P, Schilhab JC, Herd M. Reliability of a rapid latex fixation test for detecting group B streptococci in the genital tract of parturients at term. Obstet Gynecol 1989; 73:678-81.

Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: Deviations related to the development of superimposed preeclampsia

Phyllis August, MD, Tomas Lenz, MD, Kathy L. Ales, MD, Maurice L. Druzin, MD, Terri G. Edersheim, MD, J. Milton Hutson, MD, Franco B. Müller, MD, John H. Laragh, MD, and Jean E. Sealey, DSc

New York, New York

A prospective longitudinal study of 25 pregnant women (30 pregnancies) with chronic hypertension, a group prone to development of preeclampsia, was conducted to explore the relationship between the renin-angiotensin-aldosterone system and the development of superimposed preeclampsia. In women with chronic hypertension in whom preeclampsia did not develop (17 pregnancies), blood pressure decreased and the renin-angiotensin-aldosterone system was stimulated, beginning in the first trimester and continuing throughout pregnancy as found previously in normotensive pregnant women (n = 58). Plasma estradiol and progesterone levels also increased progressively. In women with chronic hypertension in whom preeclampsia developed (13 pregnancies), blood pressure decreased and the renin-angiotensin-aldosterone system was stimulated in the first trimester as in the other groups. However, later in pregnancy significant differences were observed. Blood pressure began to rise in the second trimester. Initially the renin-angiotensin-aldosterone system remained stimulated, but in the early third trimester, when preeclampsia was diagnosed, plasma renin activity and urine aldosterone excretion decreased, and atrial natriuretic factor increased. These data provide information that may be useful in the recognition of superimposed preeclampsia, and in the investigation of its pathogenesis. (AM J OBSTET GYNECOL 1990;163:1612-21.)

Key words: Chronic hypertension, preeclampsia, renin-angiotensin-aldosterone system

Preeclampsia is a common and serious disorder that occurs most often in primigravid women and women with chronic hypertension. Women in whom preeclampsia develops in the latter half of pregnancy are not easily identified early in pregnancy, and therefore there is little information on hormonal or hemodynamic alterations before clinical disease is apparent. To overcome this limitation, we studied the renin-angiotensin-aldosterone system, plasma estradiol, and progesterone levels and renal function in each trimester of pregnancy in women with chronic hypertension. Such women have an increased incidence of superimposed preeclampsia (as high as 30%)[1]; therefore longitudinal studies of these women are more likely to yeild data on early pregnancy events that precede the clinical manifestations of preeclampsia. We report the results of a longitudinal investigation of the renin system, renal function, and pregnancy hormones in 30 pregnancies complicated by chronic hypertension and compare them with 58 normotensive pregnant women studied previously by us.[2,3] Our goal was to correlate these parameters with clinical events, and to identify alterations that might clarify our understanding of pregnancy hypertension.

From the Cardiovascular Center and the Department of Obstetrics and Gynecology, Cornell University Medical College.
Supported in part by grants from the National Heart, Lung and Blood Institute of the National Institutes of Health (No. HL-18323 and No. HL-40152).
Received for publication February 23, 1990; revised July 9, 1990; accepted July 16, 1990.
Reprint requests: Phyllis August, MD, Cardiovascular Center, The New York Hospital–Cornell Medical Center, 525 East 68th St., Starr-4, New York, NY 10021.
6/1/23819

Methods

Study population. Twenty-five women with chronic hypertension were followed up longitudinally during 30 pregnancies in the outpatient offices of the Department of Obstetrics and Gynecology and the Cardiovascular Center of The New York Hospital–Cornell Medical Center. The women were seen every 2 to 4 weeks throughout pregnancy. At each visit, blood pressure measurements were obtained on three occasions with a mercury sphygmomanometer with the patient in the seated position. The fifth Korotkoff signal was recorded for diastolic blood pressure. Antihypertensive medications were adjusted to maintain blood pressure ≤150/100 mm Hg.

Chronic hypertension was defined as a blood pressure level before pregnancy or in the first trimester of >140/90 mm Hg on at least two occasions. Superim-

posed preeclampsia in women with preexisting chronic hypertension was defined as a rise in blood pressure of 30/15 mm Hg in association with either significant proteinuria (≥2+ or ≥300 mg/24 hours) or thrombocytopenia and abnormal liver function tests.[4] Patients were hospitalized when superimposed preeclampsia was either suspected or diagnosed.

The control group consisted of 58 normotensive pregnant women who were seen at least once during the first and second trimester, and two to three times (at 30, 35, and 40 weeks) during the third trimester. The selection and clinical follow-up of 44 of these normotensive women and the laboratory data of 14 of the 58 women have been previously described.[2, 3] The clinical and laboratory data of the remaining women have not been previously reported.

Infant birth weight was recorded in the delivery room. Infants were classified as small for gestational age if their birth weights were <10th percentile for a given gestational age.[5]

Laboratory studies. Venous blood and 24-hour urine collections were obtained in the first trimester, second trimester, and in the early, middle, and late third trimester when possible. Blood was drawn with patients in the seated position after they had been ambulatory.

Blood for plasma renin activity was drawn into tripotassium ethylenediamine tetraacetic acid Vacutainer blood collection tubes (Becton-Dickinson, Rutherford, N.J.) and processed at room temperature to avoid cryoactivation of prorenin.[6, 7] Samples were stored at −40° C and thawed only once before assay for plasma renin activity. Plasma renin activity was measured by radioimmunoassay of the angiotensin I generated during incubation for 3 or 18 hours at 37° C and pH 5.7 in the presence of converting enzyme and angiotensinase inhibitors, using endogenous angiotensinogen as the source of renin substrate. Results are expressed as nanograms of angiotensin I per milliliter per hour. Plasma total renin was measured as described for plasma renin activity after conversion of prorenin to renin with solid-phase trypsin.[8] Plasma prorenin was calculated by subtracting plasma renin activity from total renin. Plasma prorenin V_{max} was also calculated from the prorenin and angiotensinogen levels by use of the Michaelis—Menten equation as previously described.[9] Plasma angiotensinogen was measured as the amount of angiotensin I formed after incubation with excess human renin (6.6×10^{-2} Goldblatt units/ml).[6] The coefficient of variation of plasma renin activity and angiotensinogen is 7.6% and that of prorenin is 9.4%.

Blood for atrial natriuretic factor was drawn into chilled tripotassium ethylenediamine tetraacetic acid Vacutainer blood collection tubes and kept on ice. Samples were extracted on C18 Sep-Pak cartridges (Waters, Milford, Mass.) and radioimmunoassay was performed as previously described.[10]

Urine aldosterone was measured with a Coat-a-Count aldosterone kit (Diagnostic Products, Los Angeles). The assay was validated for use in pregnancy by showing that separation of aldosterone from other steroids by chromatography[11] did not reduce the reported values. In addition, we found that exogenous estradiol, progesterone, and human chorionic gonadotropin did not offset the radioimmunoassay. Plasma estradiol and progesterone concentrations were measured with commercially available radioimmunoassay kits (Serono Laboratories, Braintree, Mass.).

Plasma renin and urinary aldosterone were assayed as the samples were collected because these results were often used for clinical purposes. Plasma renin activity was always assayed within 2 days of collection. For the other hormones the samples for a particular patient were usually run in the same set.

Serum creatinine was assayed by multichannel automated techniques. Urinary creatinine levels were determined by an Autoanalyzer (Technicon, Tarrytown, N.Y.), and urinary sodium levels were determined by flame photometry. Urinary protein was measured by nephelometry after precipitation with trichloroacetic acid.[12]

Statistical analysis. One-way analysis of variance was used for statistical analysis of data. Blood pressure, hormones, renal parameters were compared between groups at each time period. In each group, comparisons (analysis of variance) were made between different weeks of gestation. Adjustments for multiple comparison were made with the method of Bonferroni. Tests of normality of data were performed by determining the ratio of skewness to its standard error, and the ratio of kurtosis to its standard error. For nonparametrically distributed data the Kruskal-Wallis one-way analysis of variance was used.[13]

Results

The clinical features of the 88 pregnancies are summarized in Table I.

Maternal clinical characteristics. Thirteen of the 30 hypertensive pregnancies (43%) were complicated by superimposed preeclampsia. In one woman this complication developed at 23 weeks, and in others in the third trimester. As expected, the women with superimposed preeclampsia had earlier deliveries (Table I).

Of the 13 pregnancies with superimposed preeclampsia, nine women had essential hypertension, two women had systemic lupus erythematosus (three pregnancies), and one woman had renovascular hypertension. One woman with systemic lupus erythematosus received prednisone during her entire two pregnancies to control thrombocytopenia. Two women were treated with low-dose aspirin because of high titers of anticardiolipin antibodies. Antihypertensive medications are discussed.

Table I. Description of study subjects

	Normal (n = 58)	*Chronic hypertension (n = 17)*	*Chronic hypertension with superimposed preeclampsia (n = 13)*
Maternal age (yr)	31 ± 1	32 ± 1	32 ± 1
No. of primigravid women	11/58	6/17	5/13
Gestational age at delivery (wk)	38 ± 0.4	38 ± 0.6	32 ± 1.2*
Birth weight (gm)	3430 ± 4	2616 ± 5‡	1721 ± 8†
Small for gestational age	1/58	1/17	2/13
Perinatal mortality (%)	2	0	15

Data are presented as mean ± SEM.
*$p < 0.005$, compared with normal subjects and pregnancies complicated by chronic hypertension.
†$p < 0.001$, compared with normal subjects and pregnancies complicated by chronic hypertension.
‡$p < 0.005$, compared with normal subjects.

Seventeen pregnancies in women with chronic hypertension were not complicated by preeclampsia. In 15 of these 17 women the diagnosis was essential hypertension. One women had renovascular hypertension, and one had renal hypertension (unilateral renal scar).

Infant outcome. The women with superimposed preeclampsia were delivered earlier, and their infants, as expected were smaller compared with infants of normotensive mothers and women with uncomplicated chronic hypertension ($p < 0.001$) (Table I). The infants born to mothers with uncomplicated chronic hypertension were also smaller compared with normal women ($p < 0.005$) (Table I), even though the average gestational age was the same. There were two small-for-gestational-age infants born to mothers with superimposed preeclampsia (15%). There was one intrauterine death (6%) and one perinatal death (6%) in the group with superimposed preeclampsia. Therefore the incidence of serious fetal morbidity and mortality in this group was almost 30%. There was one perinatal death caused by a cord accident and one small-for-gestational-age infant in the normotensive women. There were no perinatal deaths and only one small-for-gestational-age infant in the pregnancies of women with uncomplicated chronic hypertension.

Blood pressure. The sequential changes in systolic and diastolic blood pressure in women with chronic hypertension and normotensive women are illustrated in Fig. 1. It is evident that the blood pressure during pregnancy was higher ($p < 0.001$) in cases of chronic hypertension. It is also evident that the blood pressure decreased significantly ($p < 0.01$) during the first trimester in all hypertensive women, even in those with superimposed preeclampsia. However, in cases of uncomplicated chronic hypertension and normotension, blood pressure continued to decrease in the second trimester, reached a nadir at 28 weeks, and then rose to early pregnancy or prepregnancy levels. In contrast, blood pressure did not decrease after 10 weeks in the women in whom preeclampsia developed but remained constant or increased until the third trimester when preeclampsia was diagnosed (Fig. 1).

Renal function. Creatinine clearances (mean ± SEM) in early pregnancy were similar in normotensive women (126 ± 7 ml/min), women with uncomplicated chronic hypertension (108 ± 9 ml/min), and women with chronic hypertension in whom superimposed preeclampsia developed (129 ± 10 ml/min). Two women with chronic hypertension had proteinuria (>300 mg but <1 gm/24 hours) before and during pregnancy. The other patients had protein excretion <300 mg/24 hours.

Creatinine clearance decreased slightly only in the women in whom preeclampsia developed (129 ± 22 ml/min in the first trimester to 97 ± 13 ml/min in the third trimester, p = NS). Significant proteinuria (>300 mg/24 hours) developed in 10 of 13 women with superimposed preeclampsia in the third trimester. In the remaining three women, superimposed preeclampsia was diagnosed on the basis of a rise in blood pressure, hyperuricemia, abnormal liver function tests, and thrombocytopenia.

Renin-angiotensin-aldosterone system

Plasma renin activity. Sequential determination of plasma renin activity revealed several differences among normotensive women, women with uncomplicated chronic hypertension, and women with chronic hypertension with superimposed preeclampsia. Plasma renin activity peaked earlier in women with chronic hypertension in whom superimposed preeclampsia developed as a group and clearly later (at 32 weeks) in normotensive women and women with chronic hypertension (Fig. 2, *A*). Moreover, plasma renin activity de-

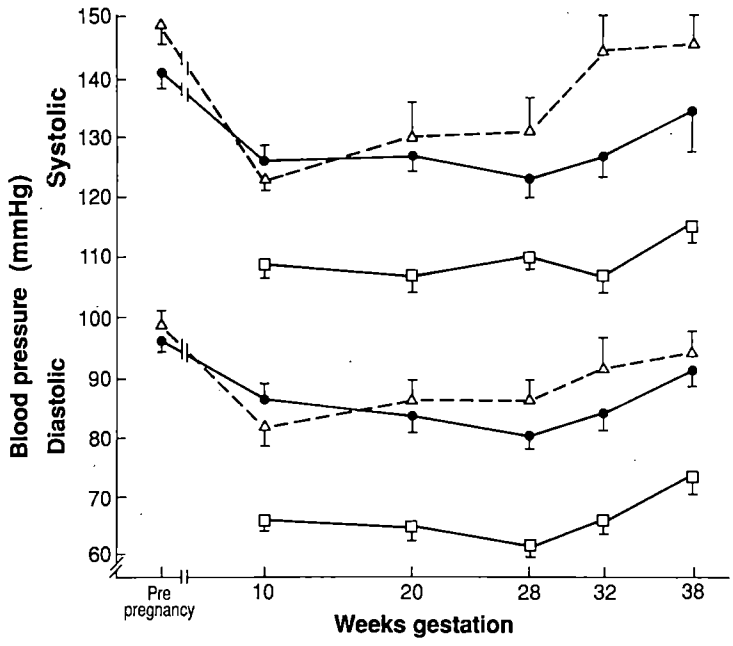


Fig. 1. Serial changes in systolic and diastolic blood pressure throughout pregnancy in normotensive women (n = 14, □——□), women with uncomplicated chronic hypertension (n = 17, ●——●), and women with chronic hypertension with superimposed preeclampsia (n = 13, △ - - - △) (mean ± SE).

creased significantly from 20 to 32 weeks in women with chronic hypertension and superimposed preeclampsia ($p < 0.01$) and increased significantly in normotensive pregnancies ($p < 0.01$). This differential profile resulted in a significantly lower plasma renin activity in women with preeclampsia compared with normotensive women at 32 weeks and not at 20 weeks. In women with uncomplicated chronic hypertension plasma renin activity remained almost constant from 20 weeks until term (Fig. 2, *A*). Late third-trimester plasma renin activity correlated with infant birth weight in the 88 pregnancies reported here ($r = 0.39$, $p < 0.01$), in accord with earlier observations made by our group.[14]

Plasma renin activity was similar in all three groups with respect to an increase in early pregnancy and a decrease in the postpartum period.

The data were reanalyzed to include only patients with essential hypertension and exclude those with renovascular hypertension, renal hypertension, or systemic lupus erythematosus, because the renin-angiotensin system might behave differently in these conditions. In these 24 pregnancies (15 with uncomplicated essential hypertension, nine with superimposed preeclampsia), the patterns of plasma renin activity were identical to those described above for the larger group. Plasma renin activity rose early in pregnancy and remained elevated until term in the women with uncomplicated essential hypertension. In contrast, in the women in whom superimposed preeclampsia developed, plasma renin activity rose early in pregnancy and then decreased after 20 weeks. Plasma renin activity in these women was significantly lower in the third trimester compared with the normotensive women and those with uncomplicated essential hypertension.

Urine aldosterone. In a fashion similar to plasma renin activity, urine aldosterone excretion differed in women with chronic hypertension with superimposed preeclampsia compared with those with chronic hypertension or normotensive women. As illustrated in Fig. 2, *B*, urine aldosterone excretion peaked earlier in women with preeclampsia. Furthermore, at 32 weeks, urine aldosterone was significantly lower in women with preeclampsia compared with normotensive women and women with uncomplicated chronic hypertension (Fig. 2, *B*). These patterns were maintained when only women with essential hypertension were analyzed (n = 24 pregnancies).

Plasma renin activity correlated with urine aldosterone in normotensive women ($r = 0.52$, $p < 0.001$). In hypertensive women the correlation between plasma renin activity and urine aldosterone was not strong ($r = 0.23$, $p = 0.05$ in uncomplicated chronic hypertension, and $r = 0.48$, $p = 0.05$ in women with preeclampsia).

Sodium excretion over 24 hours was similar in all three groups during pregnancy, except in the first trimester in the women with chronic hypertension with superimposed preeclampsia, in whom it was higher.

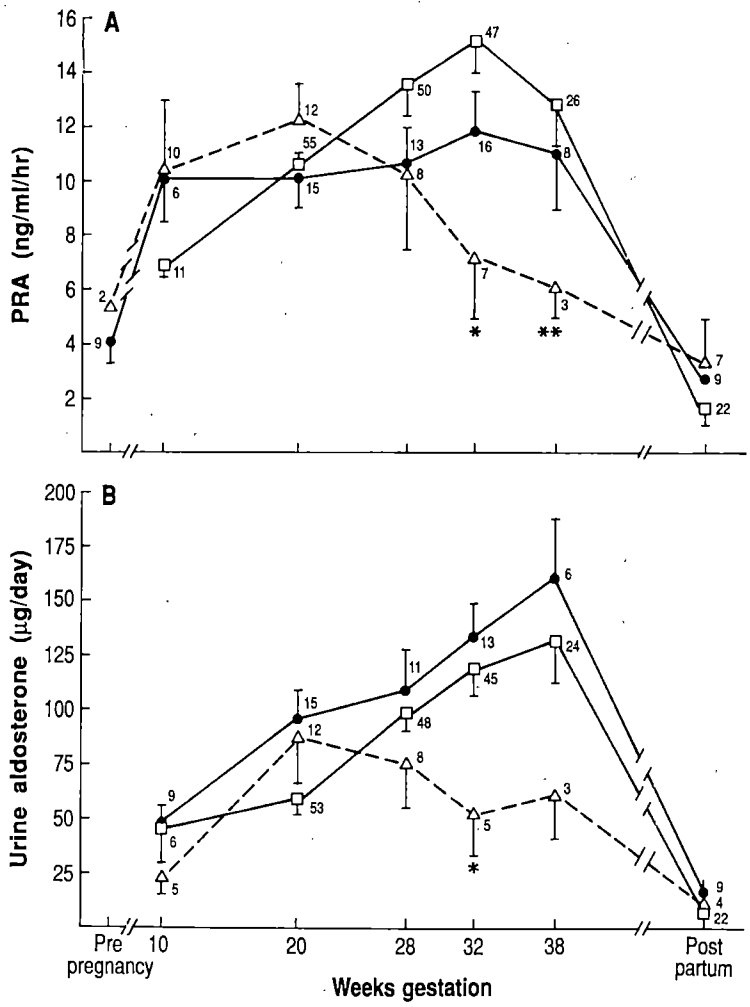


Fig. 2. Serial changes in plasma renin activity *(PRA)* **(A)** and urine aldosterone excretion **(B)** before, during, and after pregnancy in normotensive women (□——□), women with uncomplicated chronic hypertension (●——●), and women with chronic hypertension with superimposed preeclampsia (△ - - - △) (mean ± SE). *Numbers in parentheses* refer to the number of patients analyzed at each time point. Data were obtained within 2 weeks of each time point indicated on the X axis. **A,** First-trimester plasma renin activity rose significantly ($p < 0.005$) in women with uncomplicated chronic hypertension. Post partum, plasma renin activity was significantly lower than first- and second-trimester plasma renin activity in all groups. In women with superimposed preeclampsia, plasma renin activity at 32 and 38 weeks was significantly lower (*asterisk*, $p < 0.05$, *double asterisks*, $p < 0.01$) compared with the 20-week value. Plasma renin activity at 32 weeks was significantly lower ($p < 0.05$) than plasma renin activity at similar gestational age in normotensive women. **B,** Urine aldosterone was significantly lower post partum than during pregnancy in all groups. In women with superimposed preeclampsia urine aldosterone was lower ($p < 0.05$) at 32 weeks compared with urine aldosterone in normotensive women and women with uncomplicated chronic hypertension at similar gestational age.

The renin-aldosterone profile clearly distinguished, not only as a group but also in individual instances, the women with chronic hypertension with preeclampsia from those with chronic hypertension without superimposed preeclampsia. Figs. 3 and 4 show data from individual women that demonstrates the different features of the renin-aldosterone profile in women with chronic hypertension with superimposed preeclampsia compared with those with uncomplicated chronic hypertension.

Effect of antihypertensive therapy on plasma renin activity. A potential cause for lower plasma renin activity in women with chronic hypertension and superimposed preeclampsia could be treatment with drugs

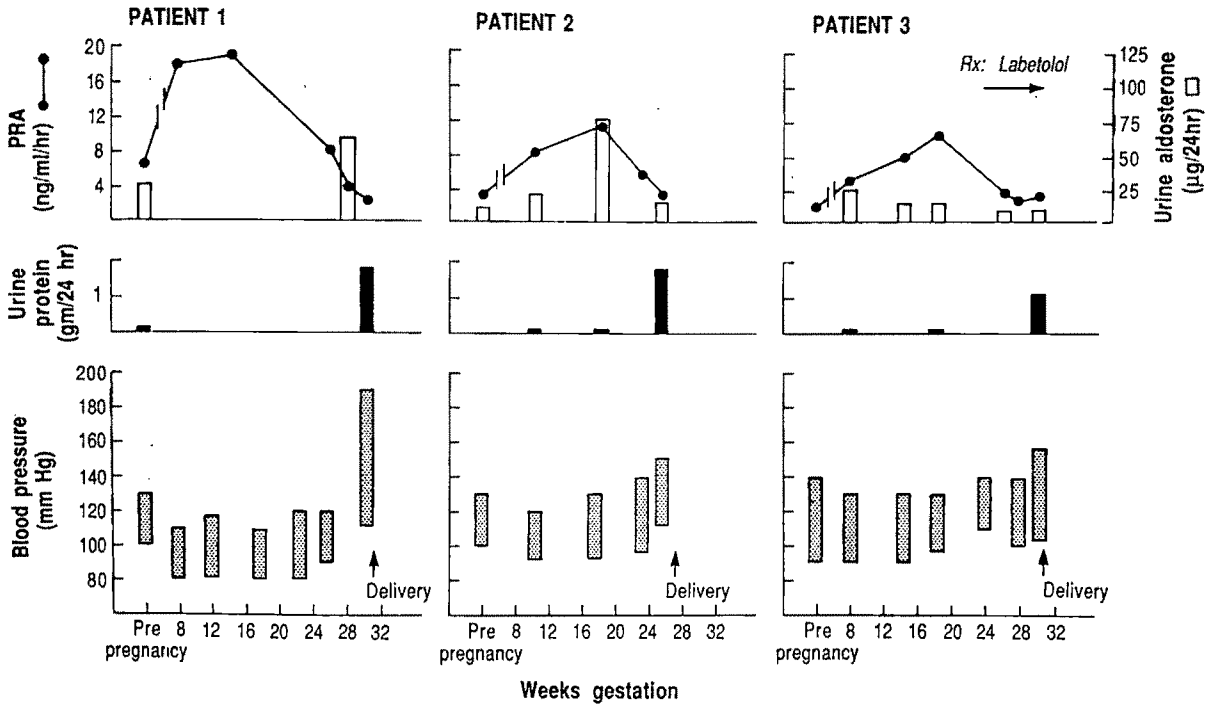


Fig. 3. Serial changes throughout pregnancy in plasma renin activity *(PRA) (solid line)*, urine aldosterone *(open bars)*, urine protein *(solid bars)*, and systolic and diastolic blood pressure in three women with chronic hypertension in whom superimposed preeclampsia developed. Patient 1 had a growth-retarded infant and patient 2 had an intrauterine death. Patient 3 was treated with labetalol during the third trimester.

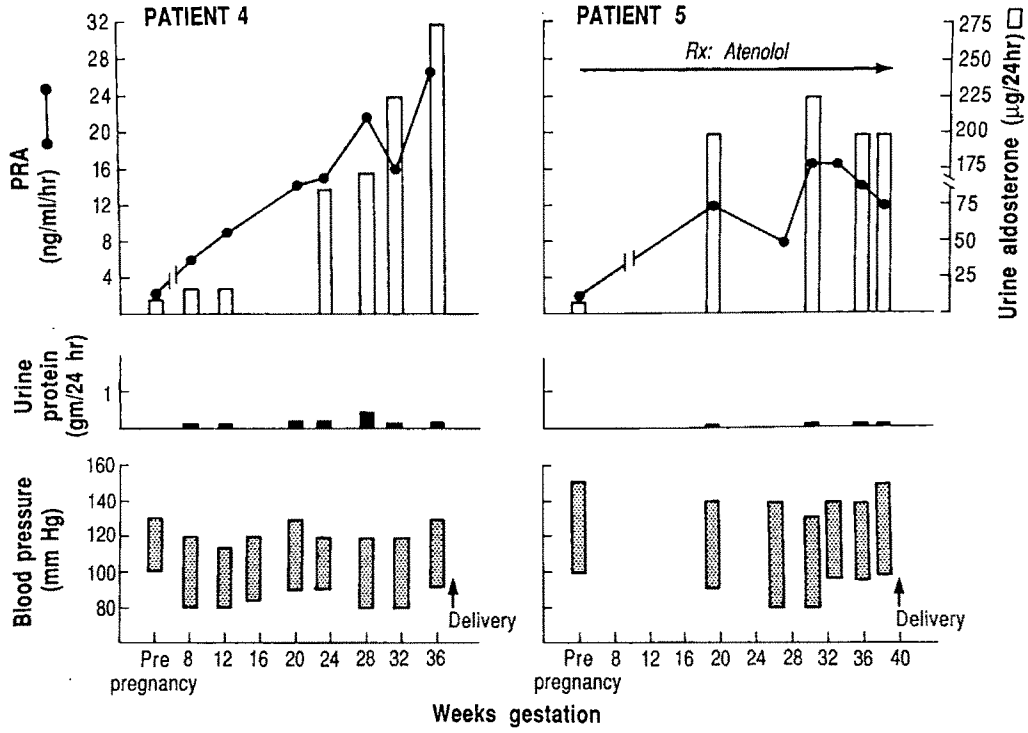


Fig. 4. Serial changes throughout pregnancy in plasma renin activity *(PRA) (solid line)*, urine aldosterone *(open bars)*, urine protein *(solid bars)*, and systolic and diastolic blood pressure in two women with uncomplicated chronic hypertension. Patient 4 received aspirin 80 mg/day, beginning at 12 weeks and continuing until term. Patient 5 was treated with atenolol during the entire pregnancy.

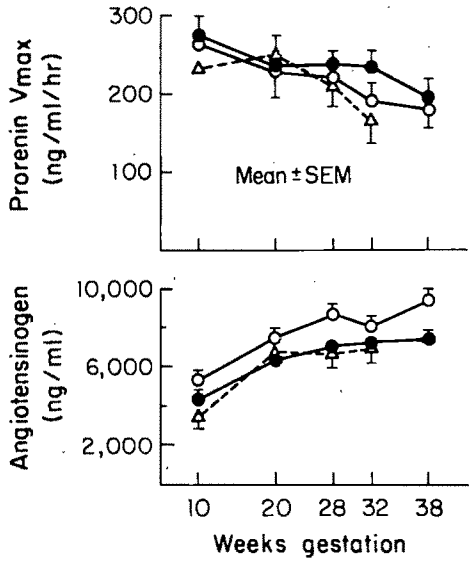


Fig. 5. Serial changes in prorenin V_{max} and angiotensinogen during pregnancy in normotensive women (○——○, $n = 58$), women with uncomplicated chronic hypertension (●——●, $n = 17$), and women with chronic hypertension with superimposed preeclampsia (△ - - - △, $n = 13$).

that affect plasma renin activity, for example, β-blockers. Of the 17 women with uncomplicated chronic hypertension, eight received antihypertensive medication, and five of eight were treated with β-blockers before the 20-week blood specimens were collected. Although medication may have resulted in lower plasma renin activity at 20 weeks in these women, by 35 weeks plasma renin activity had risen in all eight cases despite treatment.

Of the 13 women with chronic hypertension and superimposed preeclampsia, seven received antihypertensive medication. In five of these pregnancies medications were begun before the second-trimester blood sampling. Because the significant decrease in plasma renin activity occurred between the second and third trimester, it is unlikely that antihypertensive medication was responsible for the decrease in third-trimester plasma renin activity. However, it is possible that therapy may have altered the third-trimester plasma renin activity in the three women in whom treatment (one with labetalol, two with methyldopa) was begun in the second trimester. In this context, it is noteworthy that plasma renin activity rose despite therapy with labetalol and methyldopa in women with uncomplicated chronic hypertension.

Plasma prorenin, angiotensinogen. Plasma prorenin and angiotensinogen (renin substrate) levels were similar in all three groups at all times during pregnancy (Fig. 5). The prorenin level was highest early in pregnancy, and decreased slightly toward term in all three groups as previously reported in normotensive women.[7] Angiotensinogen levels increased to a similar degree in all three groups during pregnancy (Fig. 5).

Estradiol and progesterone. In normotensive women and in women with uncomplicated chronic hypertension, plasma estradiol and progesterone levels increased steadily until term (Fig. 6). Serial measurements of estradiol and progesterone were made in six of 13 women in whom superimposed preeclampsia developed (Fig. 6, *B* and *D*). The progesterone level rose in four of these women as in the other groups. In two women there was no increase after the second trimester (Fig. 6, *B*).

Estradiol levels rose throughout pregnancy in two of six women with preeclampsia. In the other four cases the levels were abnormally low in both second and third trimesters (Fig. 6, *D*).

Atrial natriuretic factor. We measured atrial natriuretic factor longitudinally in 14 women with chronic hypertension, four of whom had superimposed preeclampsia. Atrial natriuretic factor levels in six of these women have been reported previously.[15] Atrial natriuretic factor levels in the women with uncomplicated chronic hypertension ($n = 10$) remained constant throughout pregnancy and were similar to atrial natriuretic factor levels previously reported for normotensive pregnant women and nonpregnant controls.[15] In three of the four women with preeclampsia, atrial natriuretic factor rose markedly in the third trimester in association with a decrease in plasma renin activity. The third-trimester atrial natriuretic factor (mean ± SE) level in the women with preeclampsia was higher compared with the cases of uncomplicated chronic hypertension and normotensive women (67.7 ± 9 vs 16.7 ± 3.2 and 15.6 ± 1.8 fmol/ml, $p < 0.02$).

Comment

The characterization of the renin-angiotensin-aldosterone system in hypertensive pregnancy has been difficult because of the lack of longitudinal data in the same patients. Earlier cross-sectional studies, although informative, are difficult to interpret because of the wide range of normal values for plasma renin activity in pregnancy. Even in studies in which plasma renin activity was measured serially during normal and hypertensive pregnancy,[16-18] components of the renin-angiotensin-aldosterone system have not been correlated with the development of preeclampsia, or with infant outcome.

To our knowledge, this is the first report of sequential data on the renin-angiotensin-aldosterone system, atrial natriuretic factor, pregnancy hormones, and renal function in a group of high-risk pregnancies com-

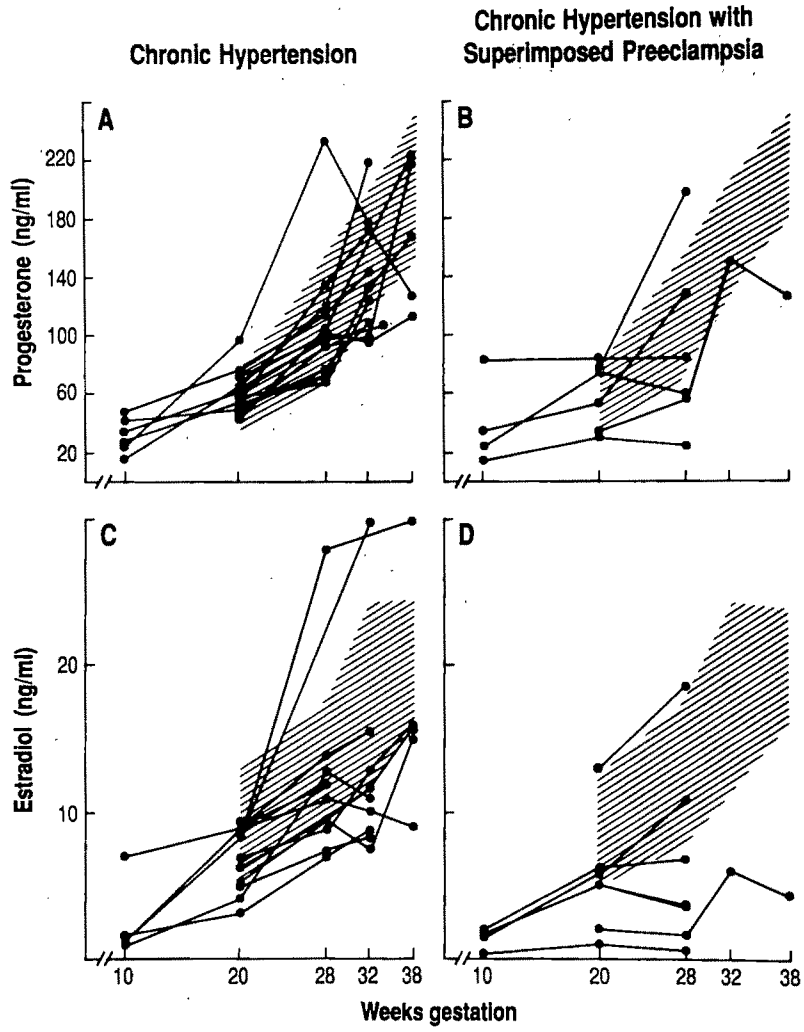


Fig. 6. Serial changes in plasma progesterone (**A** and **B**) and estradiol (**C** and **D**) in individual women with uncomplicated chronic hypertension (**A** and **C**) and those with chronic hypertension with superimposed preeclampsia (**B** and **D**). Data were obtained within 2 weeks of each time point indicated on the X axis. The *shaded area* represents the values (mean ± SD) obtained in normotensive women (n = 44).

plicated by chronic hypertension, many of which (43%) were further complicated by the development of preeclampsia. Hormonal data in 14 of the 58 normotensive controls has been previously published. A major finding of this investigation is that plasma renin activity and urine aldosterone levels decreased from previously normal (for pregnancy) levels in women with chronic hypertension as preeclampsia developed. These decreases were associated with a failure of estradiol to rise normally in the third trimester and perhaps an increase in circulating atrial natriuretic factor. Our study also demonstrates that in uncomplicated chronic hypertensive pregnancies, plasma renin activity and urine aldosterone excretion remained elevated throughout gestation, and estradiol and progesterone levels increased steadily until term in a fashion analogous to normotensive pregnancies.

Our finding of increased atrial natriuretic factor and suppressed plasma renin activity in women with preeclampsia suggests that the effective plasma volume may be expanded in this disorder. However, this notion disagrees with the observation that plasma volume (measured by Evans blue dye dilution), is lower in preeclampsia compared with normal pregnancy.[19] A possible explanation for this apparent inconsistency is that the measured decrease in plasma volume in preeclampsia does not truly reflect hypovolemia or underfilling, but rather the increased vasoconstriction has contracted the space to be filled. Alternatively, plasma atrial natriuretic factor may be increased in preeclampsia by mechanisms other than volume expansion, but still may inhibit renin directly.[20] Others have questioned the prevalent view that preeclampsia is characterized by decreased plasma volume and vasoconstriction.[21] It ap-

pears from our data that further studies may be warranted to resolve these issues.

It is most likely that the changes in plasma renin activity documented in this study are reflective of maternal renal renin secretion rather than placental renin because plasma renin activity in pregnant women responds appropriately to changes in sodium homeostasis.[22, 23] It has also been shown that increased vasodilatation during pregnancy results in stimulation of plasma renin activity.[24] Because progesterone is natriuretic,[25, 26] and there is evidence that estradiol mediates vasodilation,[27] it is possible that plasma levels of these hormones influence the renin-angiotensin-aldosterone system. Indeed, in this study, the stimulation of the renin-angiotensin-aldosterone system paralleled the rise in sex steroids, particularly in the normotensive women and women with uncomplicated chronic hypertension. Additional factors not measured in this investigation, such as vasodilatory prostaglandins, may also contribute to alterations in plasma renin activity in pregnancy.

The incidence of superimposed preeclampsia in our patients was unusually high compared with the previously reported incidence. Six of the women (20%) in our study had secondary hypertension, which may have been partially responsible for the high incidence. In addition, many of our patients were referred to our tertiary center because of previous pregnancies complicated by severe preeclampsia and fetal death. Nevertheless, this bias in our population has enabled us to investigate a small number of patients with a high percentage of cases of superimposed preeclampsia. Although it may be argued that there is a high degree of error in making a diagnosis of superimposed preeclampsia, we strictly applied the American College of Obstetricians and Gynecologists criteria[4] in classifying our patients with superimposed preeclampsia.

It may also be argued that the pathophysiologic features of preeclampsia, which is a disease of first pregnancy, may not be similar to "superimposed preeclampsia," which occurs in women who may be multiparous and who already have chronic hypertension. Therefore our findings may not be applicable to the classic primigravid woman with preeclampsia. However, it is well documented that most of the maternal and perinatal morbidity and mortality attributable to hypertensive pregnancy occurs in women with preexisting chronic hypertension in whom superimposed preeclampsia develops,[28] an observation that is supported by the data from our study and underscores the need to study such women.

In conclusion, we have demonstrated a decrease in plasma renin and urine aldosterone excretion in association with the development of superimposed preeclampsia and poor infant outcome. Although the basis for these alterations are not completely understood, our data suggest that (1) the renin-angiotensin-aldosterone system in both normotensive and hypertensive pregnancy responds to physiologic signals (e.g., changes in blood pressure, volume status) as in nonpregnant individuals and (2) alterations in placental hormones (e.g., estradiol, progesterone) and atrial natriuretic factor may be in part responsible for the alterations in the renin system in pregnancy. The possibility that inadequate suppression of the renin-angiotensin-aldosterone system may contribute to the elevation in blood pressure in preeclampsia has not been completely excluded in our study. Further studies of blocking of the renin-angiotensin-aldosterone system in hypertensive pregnancy are necessary to resolve this issue.

We thank Dr. M. Suthanthiran for his review of the manuscript.

REFERENCES

1. Chesley LC. Hypertensive disorders in pregnancy. New York: Appleton-Century-Crofts, 1978.
2. Ales KL, Norton ME, Druzin ML. Early prediction of antepartum hypertension. Obstet Gynecol 1989;73:928-33.
3. Wilson M, Morganti AA, Zervoudakis I, et al. Blood pressure, the renin-aldosterone system and sex steroids throughout normal pregnancy. Am J Med 1980;68:97-104.
4. Hughes EC, ed. Obstetric-gynecologic terminology. Philadelphia: FA Davis, 1972:422-3.
5. Lubchenco LO, Searles DT, Brazie JV. Neonatal mortality rate: relationship to birth weight and gestational age. J Pediatr 1972;81:814-22.
6. Preibisz JJ, Sealey JE, Aceto RM, Laragh JH. Plasma renin activity measurements: an update. Cardiovasc Rev Rep 1982;3:787-804.
7. Sealey JE, Wilson M, Morganti AA, Zervoudakis I, Laragh JH. Changes in active and inactive renin throughout normal pregnancy. Clin Exp Hypertens [A] 1982;4A:2372-84.
8. Sealy JE, Atlas SA, Glorioso N, Manapat H, Laragh JH. Cyclical secretion of prorenin during the menstrual cycle: synchronization with luteinizing hormone and progesterone. Proc Natl Acad Sci USA 1985;82:8705-9.
9. Sealey JE, McCord D, Taufield PA, et al. Plasma prorenin in first trimester pregnancy: relationship to changes in human chorionic gonadotropin. Am J Obstet Gynecol 1985;153:514-9.
10. Atlas SA, Volpe M, Sosa RE, Laragh JH, Camargo MJF, Maack T. Effects of atrial natriuretic factor on blood pressure and the renin-angiotensin-aldosterone system. Fed Proc 1986;45:2115-21.
11. Buhler FR, Laragh JH, Sealey JE, Brunner HR. Plasma aldosterone-renin interrelationship in various forms of essential hypertension: studies using a rapid assay of plasma aldosterone. Am J Cardiol 1973;32:554-61.
12. Schriever H, Gambino SR. Protein turbidity produced by trichloroacetic acid and sulfosalicylic acid at varying temperatures and varying ratios of albumin and globulin. Am J Clin Pathol 1965;44(6):667-72.
13. BMDP statistical software manual. Berkeley, California: University of California Press, 1985.
14. Taufield PA, Druzin ML, Edersheim TE, Sealey JE, Laragh JH. Correlation between plasma renin activity and

birthweight in hypertensive pregnancy. J Hypertens 1986;4(suppl 5):S96-S98.
15. Bond AL, August P, Druzin ML, Atlas SA, Sealey JE, Laragh JH. Atrial natriuretic factor in normal and hypertensive pregnancy. AM J OBSTET GYNECOL 1989; 160:1112-6.
16. Weinberger MJ, Kramer NJ, Petersen LP, Cleary RE, Young PCM. Sequential changes in the renin-angiotensin-aldosterone system and plasma progesterone concentrations in normal and abnormal pregnancy. In: Lindheimer MD, Katz A, Zuspan RD, eds. Hypertension in pregnancy. New York: John Wiley & Sons, 1976:263-70.
17. Broughton-Pipkin F, Symmonds EM, Lamming GD, Jadoul FAC. Renin and aldosterone concentration in pregnant essential hypertension—a prospective study. Clin Exp Hypertens Hypertens Pregnancy 1983; B2(2):255-69.
18. Gallery EDM, Stokes GS, Gyory AZ, Rowe J, Williams J. Plasma renin activity in normal human pregnancy and in pregnancy associated hypertension, with reference to cryoactivation. Clin Sci 1980;S9:49-53.
19. Chesley LC. Plasma and red cell volumes during pregnancy. AM J OBSTET GYNECOL 1972;112:440-9.
20. Atlas SA, Laragh JH. Atrial natriuretic factor and its involvement in hypertensive disorders. In: Laragh JH, Brenner BM, eds. Hypertension pathophysiology, diagnosis and management. New York: Raven Press, 1990: 861-85.
21. Easterling TR, Benedetti TJ. Preeclampsia; a hyperdynamic disease model. AM J OBSTET GYNECOL 1989; 160:1447-53.
22. Bay WH, Ferris TF. Factors controlling plasma renin and aldosterone during pregnancy. Hypertension 1979;1:410-15.
23. Brown MA, Nicholson E, Ross MR, Norton JE, Gallery EDM. Progressive resetting of sodium-renin-aldosterone relationship in human pregnancy. Clin Exp Hypertens Hypertens Pregnancy 1987; B5(3):349-74.
24. Phippard AF, Horvath JS, Glynn EM, et al. Circulatory adaptation to pregnancy—serial studies of hemodynamics, blood volume, renin and aldosterone in the baboon *(Papioi hamadrayas)*. J Hypertens 1986;4:733-79.
25. Landau RL, Lugibihl K. Inhibition of the sodium retaining influence of aldosterone by progesterone. J Clin Endocrinol Metab 1958;18:1237-45.
26. Oparil S, Ehrlich EN, Lindheimer MD. Effects of progesterone on renal sodium handling in man: relation to aldosterone excretion and plasma renin activity. Clin Sci Mol Med 1975;42:1619-31.
27. Magness RR, Rosenfeld CR. Local and systemic estradiol-17-beta: effects on uterine and systemic vasodilation. Am J Physiol 1989;256:E536-E542.
28. Lin C-C, Lindheimer MD, River P, Moawad H. Fetal outcome in hypertensive disorders of pregnancy. AM J OBSTET GYNECOL 1982;142:255-60.

A comparison of sperm antibody assays

Josef Daru, MD,* and Subbi Mathur, PhD**
Charleston, South Carolina

Cytotoxicity and Immunobead-binding immunoglobulin G assays agreed in 81% of sera from infertile men (n = 173), 74% of sera from their wives, 75% of seminal plasma, and 82% of cervical mucous samples ($p < 0.001$; χ^2 analysis of positive and negative results) when tested against sperm from the same donors. Cytotoxic and immunofluorescent IgG antibody results agreed in 79% of sera from men and women, 76% of seminal plasma, and 76% of cervical mucus ($p < 0.001$; χ^2 analysis of positive and negative results). Titers or percent binding of sperm by cytotoxicity, hemagglutination, and IgG Immunobead-binding or immunofluorescence assays in serum and secretions of husbands and wives correlated ($p < 0.001$). A significantly increased number of positive results was observed in cytotoxic and Immunobead-binding IgG assays when the husbands' and donors' sperm were used rather than donors' sperm alone. On the basis of this study we conclude: (1) that cytotoxicity, Immunobead-binding IgG, and immunofluorescent IgG sperm antibody assays correlate when sperm from the same source are used, (2) that women's isoimmunity to husbands' sperm is associated with their husbands' autoimmunity to sperm irrespective of the assay, and (3) that it is desirable to use sperm from the husbands and nonautoimmune fertile men in these assays. (Am J Obstet Gynecol 1990;163:1622-9.)

Key words: Sperm antibodies, sperm antigens, sperm cytotoxicity, sperm immobilization

Differences in basic principles of assays are the major limitations for blind studies that attempt to correlate results obtained by various sperm antibody assays and laboratories performing the same assay.[1, 2] For example, sperm agglutination assays identifying IgG2, IgG4, IgA or IgM may not wholly agree with sperm-immobilizing or cytotoxic antibody assays detecting IgG1, IgG3, and IgM in the presence of complement. It is inappropriate to compare assays that use whole semen with those using live swim-up spermatozoa free of seminal plasma.[1] The positive titers vary from laboratory to laboratory. Sperm from the same donors should be used when data from different sperm antibody assays are compared because results may vary as a result of the antigenic heterogeneity of sperm.[3, 4] Wives of autoimmune infertile men develop antibodies specifically against husbands' sperm antigens.[1, 5-9] This is attributed to special antigens present on the sperm of autoimmune infertile men.[10] It would seem appropriate to test the serum and secretions from both partners against sperm from husbands and fertile donors.

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Medical University of South Carolina.
Received for publication December 14, 1989; revised July 11, 1990; accepted July 11, 1990.
Reprint requests: Subbi Mathur, PhD, Department of Obstetrics and Gynecology, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.
**Supported by Fulbright postdoctoral fellowship, 1986 to 1987.*
***Supported by National Institutes of Health Research Career Development Award HD 00450-05.*
6/1/23740

The goals of this study were as follows: (1) to compare cytotoxicity, hemagglutination, Immunobead-binding, and immunofluorescence assays using sperm from the same donor for each run, (2) verify the incidence of sperm antibodies in both partners of infertile couples, and (3) to ascertain the need to use husbands' sperm in the assays.

Material and methods

Two hundred one infertile couples, aged 28 to 42 years and with poor postcoital test sperm motility, participated after giving informed consent. We performed double fluorochromasia cytotoxicity, Immunobead-binding, immunofluorescence, and passive hemagglutination assays in a blinded fashion. Husbands' sperm and donors' sperm were used as target cells in the cytotoxicity and hemagglutination assays of all couples and in the Immunobead-binding and immunofluorescence assays of 28 couples. Sperm from the same fertile control donors were used in each run of all assays for all study subjects.

In the cytotoxic sperm antibody assay, viable swim-up sperm labeled with diacetylfluorescein (Eastman Organic Chemicals, Rochester, N.Y.) were exposed to cytotoxic sperm antibodies in serum or secretions in the presence of complement. Counterlabeling with ethidium bromide identified the dead sperm. The end point was the dilution at which 50% of the sperm stained red. Complement, cell, negative and positive controls were regularly used for quality control. The background staining of killed sperm in the cell controls in the pres-

ence of complement alone was maintained at ≤15%. The previously established positive antibody titers were ≥32 in sera and seminal plasma and ≥80 in cervical mucus.[8] The assay yielded 0% and 6% false-positive results in fertile men and women, respectively, in a World Health Organization collaborative study.[1]

The direct and indirect Immunobead-binding assays[11, 12] were modified as follows. Sperm from fertile, type O, Rh-positive, sperm antibody–free donors were used to avoid interference by blood group antibodies; microtiter plates were used instead of test tubes (results were similar in both systems); a serum and seminal plasma dilution of 1:32 and a cervical mucus dilution of 1:40 were used instead of 1:8 used in many laboratories because dilutions ≤8 may give nonspecific and prozone reactions; Immunobead reagents were kept sterile.

A sperm pellet washed free of seminal plasma and suspended in sperm medium (Irvine Scientific, Santa Ana, Calif.) was used for the direct assay. Then 50 μl of this sperm suspension (10^6/ml) was mixed with 50 μl of 5 mg/ml Immunobeads coupled with antibodies to human IgG, IgA, or IgM (Bio-Rad Laboratories, Richmond, Calif.) and incubated for 5 to 10 minutes to detect sperm IgG, IgA, and IgM.

In the indirect Immunobead-binding assay, 50 μl of viable motile swim-up sperm (10^6/ml) from the semen of fertile donors or husbands of infertile couples was mixed with 50 μl of a 1:16 dilution of serum or seminal plasma or 1:20 dilution of cervical mucus (final dilutions of 1:32 and 1:40, respectively) in microtiter plates that were then covered and incubated for 60 minutes at 37° C. After centrifuging at 400 *g* for 15 minutes, the supernatant was aspirated, and the sperm button was resuspended in 100 μl of medium. Fifty microliters of the suspension containing 5 mg/ml Immunobeads coupled with antibodies to human IgG, IgA, or IgM was added. After a 5- to 10-minute incubation, the reaction was observed by phase-contrast microscopy at ×400. Negative and positive controls were maintained for each run. A sperm was scored positive if two or more beads were bound to its surface. Samples with ≥15% of sperm that showed Immunobead binding were classified as positive.[11, 12]

In the Immunofluorescent antibody assay, motile swim-up sperm in phosphate-buffered saline solution washed free of seminal plasma were dropped on microscopic slides and air dried for a few seconds. The serum and seminal plasma samples diluted with phosphate-buffered saline solution to 1:32 and the cervical mucus diluted to 1:80 were immediately added to sperm before the latter were killed by drying. The incubation of sperm with the test samples and secondary antibodies was done at 4° C. Fab_2 fragments of fluorescein-conjugated rabbit antihuman IgG, IgA, and IgM (Calbiochem Laboratories, Los Angeles) were used to avoid nonspecific binding and capping of antigens. The slides were examined under a fluorescent microscope. The reactions were scored as −, +, ++, or +++ depending on the intensity of fluorescence. Positive and negative serum and cell controls were maintained.[13]

In the passive hemagglutination assay, human O, Rh-positive red blood cells were coated with a sonicated sperm extract or seminal plasma from the husbands or O, Rh-positive control fertile men.[8] Serial dilutions of patient sera, seminal plasma, and cervical mucus were titrated against red cells coated with the sperm extract or seminal plasma. The test samples were positive if their antibody titers were greater than or equal to the mean + 2 SD of the fertile controls (serum and seminal plasma, ≥32; cervical mucus, ≥80).[8]

The χ^2 analyses were done to assess the similarity between sperm antibody assays in estimating the positivity or negativity of the same sample. Linear regression analysis of antibody titers conventionally expressed as $\log_2$ for cytotoxicity and hemagglutination assays and percent binding by Immunobead binding and by immunofluorescence assays was additionally performed. Only those *p* values that were significant after Bonferroni correction for multiple testing are reported.

Results

Infertile men. Sera from ≥90% of 132 men who were negative for circulating cytotoxic sperm antibodies were also negative by other assays. The positive and negative results obtained by all assays were compared by χ^2 analysis. The best agreements were between cytotoxicity and Immunobead-binding IgG in 140 of 173 (81%) serum samples ($p < 0.001$); between cytotoxicity and immunofluorescent IgG in 136 of 173 (79%) samples ($p < 0.001$); and between immunofluorescent IgG and Immunobead-binding IgG in 135 serum samples. Hemagglutinating, Immunobead-binding, and immunofluorescence assays generally yielded higher numbers of false-negative than false-positive results ($p < 0.001$; Table I).

Seventy percent or more of infertile men without cytotoxic sperm antibodies in the seminal plasma also had negative results by other assays. Results obtained by cytotoxicity and Immunobead-binding IgG were similar in 129 of 173 (75%) of seminal plasma samples, whereas cytotoxicity and immunofluorescent IgG agreed in 132 of 173 (76%) of seminal plasma samples ($p < 0.0001$). Results by cytotoxicity did not agree with those of Immunobead binding or immunofluorescent IgM. Of 30 seminal plasma samples with Immunobead-binding IgM, 25 also had Immunobead-binding IgG. Higher percentages of men had IgA and IgM sperm

Table I. Percentage agreement between cytotoxic sperm antibodies and sperm antibodies detected by hemagglutination, Immunobead-binding, and immunofluorescence assays in infertile men

Assay	*Results in relation to cytotoxicity assay*					
	Correct prediction (%)	*True positive (%)*	*True negative (%)*	*False positive (%)*	*False negative (%)*	χ^2
Serum		$n = 41$	$n = 132$			
Hemagglutination	72	5	93	7	95	0.2
Immunobead-binding						
IgG	81	39	94	6	61	28.4*
IgA	77	12	97	3	88	5.3
IgM	79	18	98	2	82	15.4*
Immunofluorescence						
IgG	79	49	88	12	51	25.5*
IgA	82	34	97	3	66	32.5*
IgM	78	20	96	4	80	11.1†
Seminal plasma		$n = 137$	$n = 36$			
Hemagglutination	31	20	72	28	80	1.1
Immunobead-binding						
IgG	75	75	72	28	25	28.4*
IgA	47	38	83	17	62	5.8
IgM	36	20	94	6	80	4.4
Immunofluorescence						
IgG	76	78	69	31	22	29.7*
IgA	57	53	75	25	47	8.4‡
IgM	50	43	78	22	57	5.2

*$p < 0.0001$.
†$p < 0.001$.
‡$p < 0.005$.

antibodies in the seminal plasma than in the serum (Table I).

Infertile women. More false-negative results were obtained with hemagglutinating, Immunobead-binding, and immunofluorescence assays than with cytotoxicity assay. Ninety percent or more of infertile women without cytotoxic sperm antibodies in the serum or cervical mucus ($n = 110$) also had negative results by the other assays. Results obtained by cytotoxicity and Immunobead-binding IgG agreed in 128 of 173 (74%) of serum and in 142 of 173 (82%) cervical mucous samples ($p < 0.0001$). Results obtained by cytotoxicity and immunofluorescent IgG agreed in 137 of 173 (79%) serum samples and in 142 of 173 (82%) cervical mucous samples ($p < 0.001$). Fourteen percent of serum and cervical mucous samples had cytotoxic and Immunobead-binding IgM sperm antibodies, whereas 24% of serum and 30% of cervical mucous samples had cytotoxic and immunofluorescent IgM sperm antibodies (Table II).

Few women had hemagglutinating, Immunobead-binding IgA and IgM and immunofluorescent IgA and IgM sperm antibodies in serum and cervical mucus (Table II).

Correlations between sperm antibody titers or percent binding by various assays. Cytotoxic sperm antibody titers ($\log_2$) correlated with percentages of sperm bound by Immunobead-binding and immunofluorescent IgG ($p < 0.001$ by linear regression analysis) in all study samples from both partners. The percentages of sperm bound by immunobead-binding and immunofluorescent IgG also correlated ($p < 0.001$).

Value of using husbands' sperm in sperm antibody assays. More men were positive for cytotoxic ($p = 0.03$ in serum and $p = 0.0002$ in seminal plasma) and Immunobead-binding IgA ($p = 0.01$ in seminal plasma only) sperm antibodies against their own sperm than against donors' sperm (Fig. 1). False-negative results were obtained in 18% of women for cytotoxic serum sperm antibodies and 4% to 29% for IgG and IgA Immunobead-binding sperm antibodies in cervical mucus, if husbands' sperm were not used in the tests (Fig. 2).

Correlation between sperm surface immunoglobulins and sperm antibodies in serum or seminal plasma ($n = 28$ couples). There was no correlation between the presence of sperm-surface immunoglobulins and cytotoxic antibodies to husbands' sperm in their serum or seminal plasma, whereas all serum samples with Immunobead-binding IgG, IgA, or IgM were from men with sperm-surface IgG, IgA, or IgM. All men with endogenous IgG, IgA, or IgM had Immunobead-binding sperm antibodies in the seminal plasma, but there were some men with Immunobead-binding an-

Table II. Percentage agreement between cytotoxic sperm antibodies and sperm antibodies detected by hemagglutination, Immunobead-binding, and immunofluorescence assays in infertile women

Assay	*Results in relation to cytotoxicity assay*					
	Correct prediction (%)	*True positive (%)*	*True negative (%)*	*False positive (%)*	*False negative (%)*	χ^2
Serum		$n = 63$	$n = 110$			
Hemagglutination	70	25	95	5	75	16.3*
Immunobead-binding						
IgG	74	46	90	10	54	29.3*
IgA	70	19	99	1	82	19.0*
IgM	68	14	99	1	86	13.2†
Immunofluorescence						
IgG	79	57	92	8	43	49.9*
IgA	75	35	96	4	65	30.7*
IgM	71	24	97	3	76	9.1*
Cervical mucus		$n = 63$	$n = 110$			
Hemagglutination	61	3	94	6	97	0.8
Immunobead-binding						
IgG	82	60	95	5	40	63.6*
IgA	68	25	91	9	75	9.6†
IgM	6	14	97	3	86	8.3‡
Immunofluorescence						
IgG	82	76	85	15	24	65.4*
IgA	73	43	91	9	57	27.2*
IgM	71	30	94	6	70	17.8*

*$p < 0.0001$.
†$p < 0.001$.
‡$p < 0.005$.

tibodies in their seminal plasma, lacking endogenous sperm immunoglobulins (Table III).

Percentages of husbands' sperm with IgG on the surface correlated with those carrying sperm-surface IgA ($r = 0.43$; $p < 0.01$, by linear regression analysis) and inversely correlated with seminal plasma cytotoxic sperm antibody titers ($r = -0.41$; $p < 0.01$). Immunobead-binding IgA on the infertile men's sperm was associated with sperm-surface Immunobead-binding IgM ($r = 0.44$; $p < 0.01$). Immunobead-binding IgA to husbands' sperm in the serum correlated with hemagglutinating antibody titers to autologous sperm ($r = 0.47$; $p < 0.01$).

Correlation between sperm antibodies in serum and secretions. Men with serum cytotoxic antibodies and Immunobead-binding IgG to autologous sperm did not always have seminal plasma antibodies to autologous sperm. Conversely, percentage binding of circulating Immunobead-binding IgA to autologous sperm correlated with percentage binding of seminal plasma IgM to autologous sperm ($r = 0.46$; $p < 0.001$) and donors' sperm ($r = 0.43$; $p < 0.01$). The presence of Immunobead-binding IgM to autologous sperm in the serum was associated with the presence of Immunobead-binding IgG to autologous and donors' sperm ($r = 0.55$; $p < 0.001$) in the seminal plasma.

Women with cytotoxic antibodies to their husbands' sperm in the serum were also positive for these antibodies in the cervical mucus ($r = 0.62$; $p < 0.001$, by linear regression analysis of $\log_2$ antibody titers). In women who had serum cytotoxic antibodies to autoimmune husbands' sperm, cervical mucous Immunobead-binding IgA to husbands' sperm was absent ($r = -0.54$; $p < 0.001$, by linear regression analysis of percent binding and $\log_2$ titers). Cervical mucous hemagglutinating antibody titers to husbands' sperm correlated with the serum Immunobead-binding IgA to husbands' sperm ($r = 0.47$; $p < 0.01$).

Correlations between the presence of sperm antibodies in both partners of infertile couples. Wives of infertile men with circulating cytotoxic and hemagglutinating antibodies to autologous sperm had circulating cytotoxic ($r = 0.63$; $p < 0.001$, by linear regression analysis of $\log_2$ titers) and hemagglutinating ($r = 0.54$; $p < 0.001$) antibodies to their autoimmune husbands' sperm. The development of cytotoxic antibodies to husbands' sperm in the cervical mucus of infertile women coincided with the presence of cytotoxic antibodies against autologous sperm in their husbands' seminal plasma ($r = 0.44$; $p < 0.01$).

Percentages of sperm with Immunobead-binding and immunofluorescent IgG antibodies to husbands' sperm in the serum and cervical mucus of infertile women correlated with percent binding of their husbands' seminal plasma Immunobead-binding IgG antibodies to autologous sperm ($r = 0.41$; $p < 0.01$). Se-

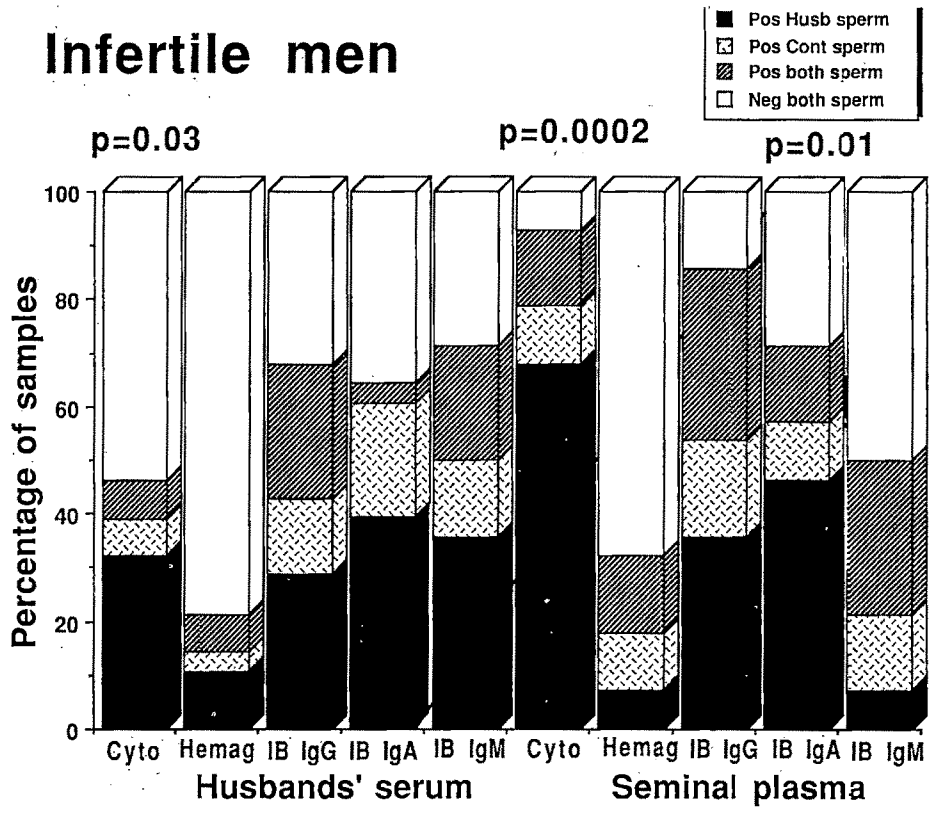


Fig. 1. Percentages of sera and seminal plasma positive for antibodies to autologous or control sperm, or both ($n = 28$).

rum immunofluorescent IgG ($r = 0.56$; $p < 0.001$) and Immunobead-binding IgM ($r = 0.50$; $p < 0.001$) were frequently present in both partners. Infertile women often had Immunobead-binding IgA ($r = 0.41$; $p < 0.01$) or IgM ($r = 0.47$; $p < 0.01$) to husbands' sperm in the serum when these antibodies were present on their husbands' sperm.

All these correlations were observed only in relation to husbands' sperm but not against donors' sperm.

Comment

This study differs from others of a similar nature[1, 2] in that freshly obtained serum, seminal plasma, and cervical mucus from infertile couples were blindly tested against sperm from the same donors by four sperm antibody assays. The data suggest that assays to identify the same isotypes of sperm antibodies correlate reasonably well when sperm from the same donor are used. Because cytotoxic sperm antibodies are primarily IgG,[14] the observed ≥75% agreement in study samples between cytotoxic, Immunobead-binding IgG, and immunofluorescent IgG assays (Tables I and II) is acceptable. However, the correlation is not perfect because the Immunobead-binding and immunofluorescent antibody assays are affected by the specificity, sensitivity, and variability among batches of commercially available antibodies to human immunoglobulin isotypes[15] and because these assays are not yet designed to identify specific subclasses of IgG-mediating cytotoxicity. False-positive results by cytotoxicity assay could hardly have contributed to the discrepancy since samples positive by cytotoxicity assay were also positive by immunofluorescence or Immunobead-binding assays, or both. In addition, ≥90% of samples negative by cytotoxicity were negative by other assays, showing a low incidence of false-negative results by this assay. False-negative results were observed in Immunobead-binding and immunofluorescent IgG assays (Tables I and II). Motile spermatozoa often may avoid being bound with the Immunobeads in contrast to sluggish spermatozoa. Passive hemagglutination and Immunobead-binding and immunofluorescent IgA antibody assays correlated, leading to the surmise that sperm-agglutinating antibodies are essentially IgA.

The study results also emphasize the use of husbands' sperm in sperm antibody assays and support previous reports by our group and others. The cytotoxic sperm antibody assay appears to be most sensitive to antigenic differences between sperm of autoimmune infertile men and nonautoimmune fertile men (Figs. 1 and 2).

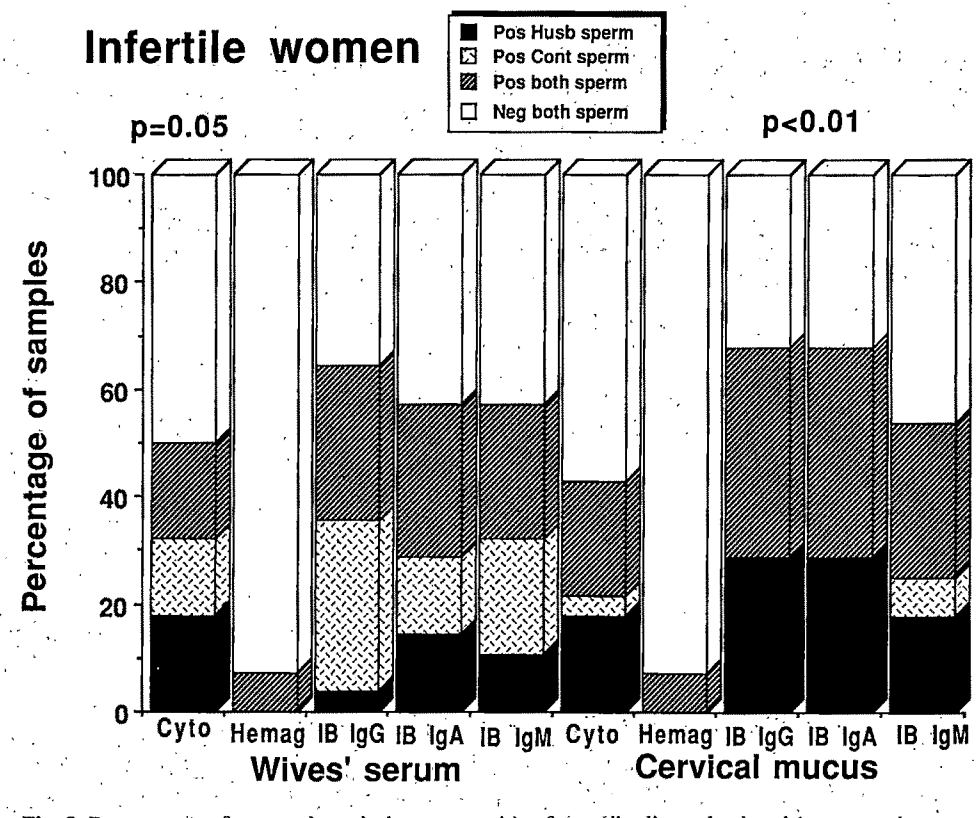


Fig. 2. Percentages of sera and cervical mucus positive for antibodies to husbands' or control sperm, or both (n = 28).

Table III. Percentage of men positive for sperm-surface immunoglobulins and sperm antibodies in the serum or seminal plasma, or both

	Positive for sperm surface				
	IgG, IgA, and IgM (n = 4)	*IgG and IgA* (n = 3)	*IgG* (n = 6)	*IgA* (n = 1)	*None* (n = 14)
Cytotoxic					
Serum	50	33	17	0	43
Seminal plasma	50	33	100	100	71
Immunobead-binding					
IgG					
Serum	100	100	100	100	0
Seminal plasma	100	100	100	100	71
IgA					
Serum	75	100	67	100	0
Seminal plasma	75	100	67	100	14
IgM					
Serum	50	100	33	100	43
Seminal plasma	100	100	67	100	0
Hemagglutination					
Serum	75	100	0	100	0
Seminal plasma	75	66	0	100	0

These differences may be attributed to the documented presence of special antigens with molecular weights of 30, 43, 58, and 78/80 kd on the sperm of autoimmune infertile men and reactive against IgG.[10] It is possible that these antigens are not involved in eliciting IgM or IgA responses measured by sperm agglutination assays, including passive hemagglutination, Immunobead-binding, or immunofluorescent IgA or IgM (supported by Western blot analysis data from this laboratory).

It may be argued that the presence of endogenous

immunoglobulins on the sperm surface of autoimmune infertile men may lead to false-positive or enhanced results by cytotoxicity and Immunobead-binding assays. The following data refute this point of view: (1) presence of serum or seminal plasma cytotoxic sperm antibodies or seminal plasma Immunobead-binding IgG was independent of the presence of endogenous immunoglobulins (Table III); (2) fewer swim-up sperm used in cytotoxicity and indirect Immunobead-binding assays (≤15% binding) have endogenous immunoglobulins than the entire sperm population pelletted for the direct Immunobead-binding assay (35% to 100%); (3) cytotoxicity assay requires that sperm cell controls without added serum or secretions must have a viability ≥85% for the assay to be valid. The observed differences are therefore a result of sperm antigenic heterogeneity.[5-10] Qualitatively, antibodies against autoimmune men's sperm inhibit sperm motility in the presence of native complement.[16]

Immunobead-binding IgG on the surface of husbands' sperm was associated with endogenous IgA and IgM. Because sperm-surface IgM was also associated with serum Immunobead-binding IgG and serum and seminal plasma IgA sperm antibodies in these men (Table III), it is possible that nonspecific immune responses triggered by infections are detected by the Immunobead-binding assay. In the earlier World Health Organization study, results obtained by Immunobead-binding and agglutination assays correlated with the presence of antibodies to *Mycoplasma* organisms.[17] In this study men with sperm-surface Immunobead-binding IgA were also positive for circulating Immunobead-binding IgM and hemagglutinating sperm antibodies. The primary immune response to microorganisms or viruses in the genital mucosa is IgM- and IgA-mediated and leads to a secondary IgG response. That fewer men were positive for serum Immunobead-binding or immunofluorescent IgM than for seminal plasma IgM sperm antibodies suggests a localized IgM immune response to infection. IgM is known to be present, albeit at low levels, in fresh seminal plasma[18] and is more susceptible to native proteolytic enzyme action than IgG or IgA.[19] Fresh seminal plasma was used in this study and may account for the increased level of detection of IgM in comparison with laboratories that use stored frozen seminal plasma samples.

Our data also suggest that development of autoimmunity to sperm in infertile men may be heterogeneous. Infertile men with cytotoxic, Immunobead-binding, and immunofluorescent IgG antibodies to autologous sperm in the serum and seminal plasma may be genetically prone to produce these antibodies, whereas after infections other men may develop transient sperm antibodies in the seminal plasma but not in the serum. The sperm motion characteristics of the latter are normal in contrast to the critically affected sperm motion characteristics of infertile men with true autoimmunity to sperm in serum and seminal plasma.[20]

Unlike their husbands, the wives of autoimmune infertile men had sperm antibodies in both serum and cervical mucus. Women exposed to the unique sperm antigens of their autoimmune infertile husbands[10] or to an excess of antigens generated through degradation of infected sperm in their husbands may have cervical mucous sperm antibodies. Circulating sperm antibodies may develop in these women as a result of transudation of cervical mucous sperm antibodies or postcoital soluble sperm antigens from their husbands. Serum Immunobead-binding IgA to husbands' sperm developed in women whose husbands had sperm-surface Immunobead-binding IgA and IgM. The latter couples might have sperm antibodies as a result of infections in the husbands.

It has been shown[1, 5, 7-9] that cytotoxic, immobilizing, and hemagglutinating sperm antibodies in the serum and secretions of both partners in infertile couples correlate with each other; this was substantiated by this study. In most instances this correlation was in immune responses of both partners, specifically against sperm from autoimmune infertile husbands, reemphasizing the need to use husbands' sperm in these sperm antibody assays.

Sperm-immobilizing and cytotoxic antibodies are sperm specific and may be better correlated with infertility than sperm-agglutinating antibodies.[21] Immunobead-binding and immunofluorescence assays identify the isotypes of sperm-reactive antibodies.[11, 12, 15] The combination of an assay to evaluate sperm immobilizing function and another assay that enumerates the isotype of immunoglobulins appears preferable to use of a single assay because such an approach yields information on the immunoglobulin isotype and its functional significance. For example, cytotoxic sperm antibodies, singly or in combination with Immunobead-binding or immunofluorescent IgG, denote the presence of true immunity to sperm, whereas the presence of Immunobead-binding or immunofluorescent IgG, IgA, or IgM in absence of cytotoxic sperm antibodies may imply nonspecific infection-related antibodies. Whereas corticosteroid therapy is desirable for the former, antibiotic therapy would seem appropriate for the latter.

It is concluded (1) that cytotoxicity, Immunobead-binding (IgG), and immunofluorescence (IgG) assays are in agreement when sperm from the same donors

are used, (2) that a significant incidence of false-negative results could occur especially in infertile men, when husbands' sperm are not used; (3) that the women's isoimmunity to sperm is associated with their husbands' autoimmunity to sperm.

We thank H. Oliver Williamson, MD, Chief, Section of Reproductive Endocrinology and Infertility and Vice Chairman, Obstetrics and Gynecology Department, for providing the clinical samples and reviewing the manuscript.

REFERENCES

1. Bronson RA, Cooper GW, Rosenfeld DL, et al. Antisperm antibodies detected by agglutination, immobilization and spermatoxicity tests and by immunobead-binding technique. J Reprod Immunol 1985;8:279.
2. Mettler L, Czuppon AB, Alexander N, et al. Antibodies to spermatozoa and seminal plasma antigens detected by various enzyme-linked immunosorbent (ELISA) assays. J Reprod Immunol 1985;301.
3. Lynch DM, Howe SE. Antibody binding specificity to donor sperm in sera from infertile patients. Am J Reprod Immunol Microbiol 1987;13:104.
4. Chong X, Anderson DJ. Differences in the immunogenicity indicating polymorphism of sperm antigens from mice of different inbred strains. J Reprod Immunol 1987;11:167.
5. Mathur S, Williamson HO, Genco PV, Koopman WR, Rust PF, Fudenberg HH. Sperm immunity in infertile couples: antibody titers are higher against husbands' sperm than to sperm from controls. Am J Reprod Immunol 1983;3:18.
6. Mathur S, Pathak S, Kandel A, Ziegler J, Rust PF, Williamson HO. Leukocyte migration inhibition factor (LIF) to sperm from autoimmune men in infertile couples. J Immunol 1986;136:4444.
7. Witkin SS, Berkeley A, David SS, Goldstein M, Toth A. Antibodies to husband sperm but not to donor sperm in sera of infertile couples [Abstract P-101]. In: Program supplement, Forty-second annual meeting of the American Fertility Society and The Canadian Fertility and Andrology Society, September 27 to October 2, 1986, Toronto, Ontario, Canada. Birmingham: American Fertility Society, 1986:25.
8. Mathur S, Williamson HO, Derrick FC, Melchers T, Smith CL, Fudenberg HH. A new microassay for spermocytotoxic antibodies: comparison with passive hemagglutination assay of antisperm antibodies in couples with unexplained infertility. J Immunol 1981;126:905.
9. Menge AC, Medley NE, Mangione CM, Dietrich JW. The incidence and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. Fertil Steril 1983;38:439.
10. Mathur S, Chao L, Goust JM, et al. Special antigens on sperm from autoimmune infertile men. Am J Reprod Immunol Microbiol 1988;17:5.
11. Bronson RA, Cooper GW, Rosenfeld DL, Witkin SS. Detecting spontaneously occurring sperm-directed antibodies in infertile couples by Immunobead-binding and enzyme-linked immunosorbent assay. Ann NY Acad Sci 1984;1984:438.
12. Clarke GN, Stojanoff A, Cauchi MN, Johnston WIH. The immunoglobulin class of antispermatozoal antibodies in serum. Am J Reprod Immunol Microbiol 1985;7:143.
13. Hansen KB, Rebbe H. Immunofluorescent studies on human spermatozoa. IV. Comparison of antigens in spermatozoa from fertile men and results of semen analysis. Int J Fertil 1973;18:101.
14. Mathur S, Williamson HO, Genco PV, Nesmith D, Rust PF, Fudenberg HH. Cytotoxic antibodies to lymphocytes and sperm immunity. J Reprod Immunol 1983;5:89.
15. Bronson RA. Immunobead-binding: present and future uses. In: Mathur S, Fredericks CM, eds. Perspectives in immunoreproduction: conception and contraception. Washington DC: Hemisphere Publishing, 1988;102.
16. Mathur S, Rosenlund K, Barber M, Caldwell J, Williamson HO. Studies on sperm survival and motility in the presence of cytotoxic sperm antibodies. Am J Reprod Immunol Microbiol 1988;17:41.
17. Mathur S, Genco PV, Moller B, Mardh PA. Antibodies to microbial, leukocyte and organ autoantigens. J Reprod Immunol 1985;8:353.
18. Azim AA, Fayad S, Fattah AA, Habib M. Immunologic studies of male infertility. Fertil Steril 1978;30:426.
19. Tjokronegoro A, Sirishinha S. Degradation of immunoglobulins by secretions of human reproductive tracts. J Reprod Fertil 1974;38:221.
20. Mathur S, Barber M, Carlton M, Ziegler J, Rust PF, Williamson HO. Motion characteristics of spermatozoa from men with cytotoxic sperm antibodies. Am J Reprod Immunol Microbiol 1986;12:87.
21. Isojima S, Koyama K. Sperm antibodies detected by sperm immobilization test. In: Mathur S, Fredericks CM, eds. perspectives in immunoreproduction: conception and contraception. Washington DC: Hemisphere Publishing, 1988:61.

Extensive vulvar and vaginal varicella necessitating abdominal delivery

Cynthia K. Sites, MD, David M. Sherer, MD, David L. Gandell, MD, and James R. Woods, Jr., MD
Rochester, New York

Maternal varicella occurs in fewer than five per 10,000 pregnancies in the United States. A case is reported in which markedly painful, extensive vulvar and vaginal ulcers prevented cervical examination, and cesarean section was performed with the patient under general anesthesia. (AM J OBSTET GYNECOL 1990;163:1630-1.)

Key words: Varicella-zoster, chickenpox, abdominal delivery

The rash of varicella-zoster usually begins on the face or scalp and progresses rapidly to the trunk, often sparing the extremities. Lesions begin as macules and papules and evolve to pustules and crusts, with completion of the cycle in 8 to 12 hours. Lesions can also develop in mucous membranes, including the mouth and vagina. These appear as vesicles that rupture quickly, leaving shallow ulcers. We report a case where a patient exposed to varicella early in pregnancy received varicella-zoster immune globulin but extensive vulvar and vaginal varicella at term necessitated abdominal delivery.

Case report

A 32-year-old white woman, gravida 3, para 2, was admitted to the labor and delivery suite with mild irregular contractions at 41⅐ weeks' gestation. A nonstress test that day was reactive, but a marked decrease in amniotic fluid was noted by ultrasonography.

At 7 weeks into the current gestation, she had received five vials of varicella-zoster immune globulin 9 days after exposure to chickenpox. Gestational diabetes was well controlled by diet. Her history was negative for genital herpes.

Eight days before admission, she first noted fever, malaise, and occasional coughing. The next day fever, shaking chills, and a rash typical of varicella were seen. To her knowledge, she had not been reexposed to varicella.

On admission she was afebrile, with blood pressure 110/76 mm Hg and pulse 80 beats/min. Examination of the mouth revealed a few ulcers. Results of heart and lung examination were normal. Abdominal examination revealed a fundal height of 35 cm, with a vertex-presenting fetus that had a normal fetal heart rate. Extremities, face, trunk, and abdomen were covered with lesions that were mostly crusted but in various stages of healing (Fig. 1). Cervical examination was intolerable for the patient because of multiple painful vulvar and vaginal ulcers (Fig. 2). On initial examination the cervix was 1 to 2 cm dilated and 50% effaced, and the vertex was at the −3 station. A chest x-ray film showed no evidence of varicella pneumonia.

Because of the gestational age in conjunction with olighohydramnios in a patient with gestational diabetes, delivery was elected. Options for delivery were discussed with the patient and included intravenous oxytocin induction of labor with anesthesia, waiting for more healing before attempting vaginal delivery, or cesarean section. Members of anesthesiology, perinatology, and neonatology departments were consulted. Because of the skin lesions on the back, spinal or epidural anesthesia was thought to be a high-risk factor for meningitis. Since even gentle touching of the vaginal lesions caused great discomfort for the patient, the decision was made to perform a cesarean section while she was under general anesthesia. A female infant weighing 3205 gm was delivered with Apgar scores of 7 and 8 at 1 minute and 5 minutes, respectively.

The infant did not receive varicella-zoster immune globulin, because the pediatric infectious disease team thought that, because maternal symptoms appeared more than 5 days before delivery, maternal antibodies had formed and had been passively transferred to the infant. The mother and infant were both discharged in good health 5 days after delivery. In the infant a mild rash suggestive of varicella developed several days later.

Comment

It is uncertain how our patient contracted chickenpox. Enders[1] has shown that varicella-zoster immune

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Strong Memorial Hospital, The University of Rochester School of Medicine and Dentistry.
Received for publication April 27, 1990; accepted May 11, 1990.
Reprint requests: David M. Sherer, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Strong Memorial Hospital, Box 668, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Rochester, NY 14642.
6/1/22422

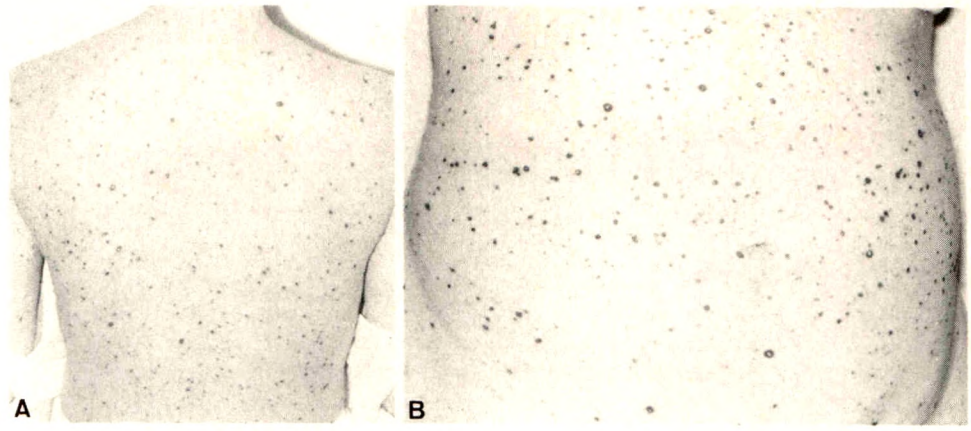


Fig. 1. Extensive varicella lesions on back **(A)** and abdomen **(B)**.



Fig. 2. Extensive perineal varicella lesions.

globulin, when given within 96 hours of exposure, is protective in 80% of cases in 25 seronegative patients. Varicella developed in approximately 90% of 18 women who did not receive varicella-zoster immune globulin. Nine patients who received varicella-zoster immune globulin >96 hours after chickenpox exposure had an attenuated form of the disease. Because our patient received varicella-zoster immune globulin >96 hours after exposure at 7 weeks' gestation, it is possible that varicella-zoster immune globulin delayed expression of the disease. Alternatively, she could have been reexposed unknowingly.

To our knowledge, this is the first reported cesarean section performed because of extensive vulvar and vaginal varicella ulcerations. We do not suggest that peripartum maternal varicella is in itself an indication for cesarean section for fetal or maternal reasons. However, this case represents a rare maternal genital manifestation of varicella precluding vaginal delivery because of exquisitely painful lesions in a patient in whom delivery was deemed necessary, with regional anesthesia contraindicated. Steyn and Odendaal[2] reported a case of a primigravid woman with varicella pneumonia at 33 weeks' gestation with repetitive late fetal heart rate decelerations. Labor was suppressed, with subsequent improvement of maternal and fetal conditions. The patient subsequently underwent a cesarean section because of breech presentation with good outcome.

REFERENCES

1. Enders G. Management of varicella-zoster contact and infection in pregnancy using a standardized varicella-zoster ELISA test. Postgrad Med J 1985;61:23-30.
2. Steyn DW, Odendaal HJ. Fetal resuscitation in a patient with varicella pneumonia and preterm labor. Int J Gynecol Obstet 1989;30:171-5.

The role of fetal membranes in regulating production, transport, and metabolism of prostaglandin E_2 during labor

John A. McCoshen, PhD,[a] Dennis R. Hoffman, PhD,[b] Jeremy V. Kredentser, MD,[a] Carolina Araneda,[a] and John M. Johnston, PhD[b]

Winnipeg, Manitoba, Canada, and Dallas, Texas

The level of prostaglandin E_2, a potent stimulator of myometrial contractions, increases in amniotic fluid during labor. We tested whether amniotic prostaglandin E_2 crosses the reflected fetal membranes to the myometrium during labor. Disks of amnion, chorion-decidua, and amnion chorion-decidua from 10 term pregnancies before labor and nine vaginal deliveries after normal spontaneous labor were incubated in two-sided perfusion chambers in the absence or presence of 1 μmol/L indomethacin, or in the presence of a bolus of prostaglandin E_2 and radiolabeled prostaglandin E_2 added to the fetal chamber. Net amniotic prostaglandin E_2 increased significantly ($p = 0.003$) after labor. However, there was no significant difference in prostaglandin E_2 concentrations on the maternal side of amnion chorion-decidua between labor conditions. High-pressure liquid chromatography identified the transport of intact tritiated prostaglandin E_2 across amnion but not across chorion-decidua or amnion chorion-decidua in which prostaglandin E_2 was completely metabolized. Because of 15-hydroxyprostaglandin dehydrogenase activity in chorion, we suggest that amniotic prostaglandin E_2 transport to the myometrium is impaired. Thus the increase in prostaglandin E_2 production that occurs in amnion tissue after labor commences does not result in an increase in prostaglandin E_2 concentration on the myometrial side of the fetal membranes in the in vitro preparation. (Am J Obstet Gynecol 1990;163:1632-40.)

Key words: Fetal membranes, prostaglandin E_2 metabolism, labor

Prostaglandins of fetal origin are believed to be important factors in regulating myometrial contractions and the events of labor.[1, 2] During labor the substantial increase in the concentration of prostaglandin E_2 (PGE_2) in amniotic fluid[1, 3] appears to result from an elevated production of PGE_2 by amniotic membrane lining the gestational sac,[4, 5] as a result of increased activities of the enzymes responsible for release of arachidonic acid[2] and prostaglandin synthesis.[2] This progressive accumulation of PGE_2 in amniotic fluid poses a potential for increased availability of PGE_2 to myometrium by translocation across the fetal membranes.[3, 6] However, this concept is complicated by the observations that (1) intact reflected membranes, consisting of adherent amnion plus chorion-decidua obtained from women after the spontaneous onset of labor, display increased PGE_2 output on the amniotic surface but not on the maternal surface facing the myometrium,[7] and (2) chorion laeve lying between amnion and decidua possesses high 15-hydroxyprostaglandin dehydrogenase activity capable of metabolizing PGE_2.[1] Decidual tissue also has the capacity to snythesize, secrete, and metabolize PGE_2 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)[1, 2] and appearance of PGE_2 on the maternal side of the fetal membranes may not necessitate solely an amniotic source. This study was performed to determine the translocation of PGE_2 from fetal to maternal compartments and to examine the metabolism of the prostanoid during transit.

Material and methods

Placentas were collected from 10 clinically normal women at term (38 to 40 weeks) after delivery by elective repeat cesarean section but before labor onset (prelabor). A second group of samples was obtained from nine normal women who were delivered vaginally at term following the spontaneous onset of labor (postlabor). Tissues were immediately taken to the laboratory and rinsed in cold (4° C) phosphate-buffered saline solution (pH 7.4) to remove residual blood. After reflected membranes were severed from the placenta, 12

From the Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba,[a] and Departments of Biochemistry, Pediatrics, and Obstetrics-Gynecology, The Cecil H. and Ida Green Center for Reproductive Biology Sciences, The University of Texas Southwestern Medical Center at Dallas.[b]

Supported by a grant MA7722 from the Medical Research Council of Canada, National Institutes of Health, grant 5-PO5-HD11149, and the Robert A. Welch Foundation, Houston. Personal support for C.A. was kindly provided by the Manitoba Health Research Council. D.R.H. was a recipient of a postdoctoral fellowship by the Chilton Foundation.

Received for publication December 21, 1989; revised July 20, 1990; accepted July 20, 1990.

Reprint requests: John A. McCoshen, PhD, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, 59 Emily St., Winnipeg, Manitoba, Canada R3E 1Y9.

6/1/24036

discs of intact membrane (approximately 8 cm^2) were cut from each tissue. Subsequently, different membrane combinations were prepared to include four disks each of amnion, chorion-decidua, and intact membrane.

Reflected fetal membrane is a composite of amnion fused to smooth chorion (chorion laeve). After separation from the uterus, a thin layer of decidua usually remains adherent to the maternal surface of chorion. Thus the term *chorion-decidua* best describes the chorionic layer of the membranes after this separation from the uterus. The amniotic component of reflected membranes is fused to the chorion-decidua. Thus, for purposes of simplifying the terminology, "intact" membranes will be used hereafter to designate amnion plus chorion-decidua.

Each disk of tissue was mounted inside a dual-compartment perfusion chamber described previously.[7] The chambers were checked over a 10-minute period to ensure that the preparations were free of leaks. During incubation constant circulation and continuous aeration with filtered humidified air were maintained in the chambers. Synthetic amniotic fluid[8] at pH 7.4 was added to the chambers (9 ml each) on the fetal and maternal sides of suspended tissue and preincubated at 20° C for 2 hours with a change of medium after each hour. By this procedure endogenous PGE_2 secretion caused by the initial trauma was eluted and the preparation was stabilized.[9] After the second change of medium, the chambers were placed in a water bath at 37° C and allowed to equilibrate to ambient temperature over 15 minutes. The time point after equilibration was designated as 0 hours. The experimental regimen for each membrane type from each patient included control membranes incubated in medium alone, membranes incubated in the presence of 1 μmol/L indomethacin, membranes in which a bolus of PGE_2 (final concentration = 6.0 nmol/L) was added to the fetal chamber, and a fourth membrane incubated in medium containing 1 μmol/L indomethacin plus a bolus of PGE_2 on the fetal side. Thus for each membrane type from each patient determinations were made for PGE_2 production, inhibition of PGE_2 production, PGE_2 transport from the fetal to maternal surfaces, and PGE_2 transport during inhibition of PGE_2 production. Table I summarizes the variables used in the experimental design.

Samples of 0.5 ml were removed from the fetal and maternal chambers at 1 and 2 hours of the preincubation period and at 0, 2, and 4 hours from initiation of the experiment. PGE_2 values were corrected for the fixed loss of media volume as a result of sampling. Radioimmunoassay (RIA) of PGE_2 was performed as previously described.[10] The interassay variation was 10% and the intraassay variation was <8%.

Table I. Variables used in the experimental design testing the translocation and restrictions of translocation of PGE_2 across reflected membrane from term deliveries

Delivery types
Cesarean section (prelabor)
Spontaneous vaginal delivery (postlabor)
PGE_2 on fetal and maternal membrane sides
Membranes
Intact (amnion plus chorion-decidua)
Chorion-decidua
Amnion
Sampling times
0 hr
2 hr
4 hr
Treatments
Control
Plus 1 μmol/L indomethacin (both sides)
Plus PGE_2 bolus (fetal side only)
Plus indomethacin (both sides) and PGE_2 bolus (fetal side only)

Separate experiments were performed on normal tissues obtained from each of five women before labor and after labor, whereby 0.21 pmol of [^{3}H]PGE_2 (0.03 μCi, >98.4% purity) was added to the fetal side of either the amnion, chorion-decidua, or intact membranes at 0 hours after preequilibration. Samples (0.1 ml) were removed from the fetal and maternal sides at 0, 2, and 4 hours of incubation at 37° C and total counts per minute were assessed by liquid scintillation counting. Comparisons were then made between the results of RIA for PGE_2 and the extent to which tritiated compound(s) appeared in the maternal compartment.

Additional aliquots of media were used for determination of radioactivity in various prostaglandins and their metabolites. The individual prostanoids were separated by high-pressure liquid chromatography (HPLC) according to Campbell and Ojeda.[11] Briefly, PGE_2, 15-keto-PGE_2, and 15-keto-dihydro-PGE_2 (5 μg each) carrier was added to 3 to 5 ml of sample; the samples were acidified and passed through a Bondelut octadecylsilyl column. The prostaglandins were eluted with ethyl acetate. The prostaglandins were then fractionated on a Dupont 850 liquid chromatograph fitted with an Alltech Econosphere C18 reverse-phase column (250 mm × 4.6 mm × 5 μm) eluted with 68% phosphoric acid (25 mmol)/L acetonitrile for 40 minutes and a linear gradient to 100% acetonitrile for 20 minutes. Optical density was monitored at 192 nm; radioactivity was quantitated with a Radiomatic Flo-One radioactivity flow detector.

All results were statistically evaluated by a general linear model analysis of variance, individual paired and

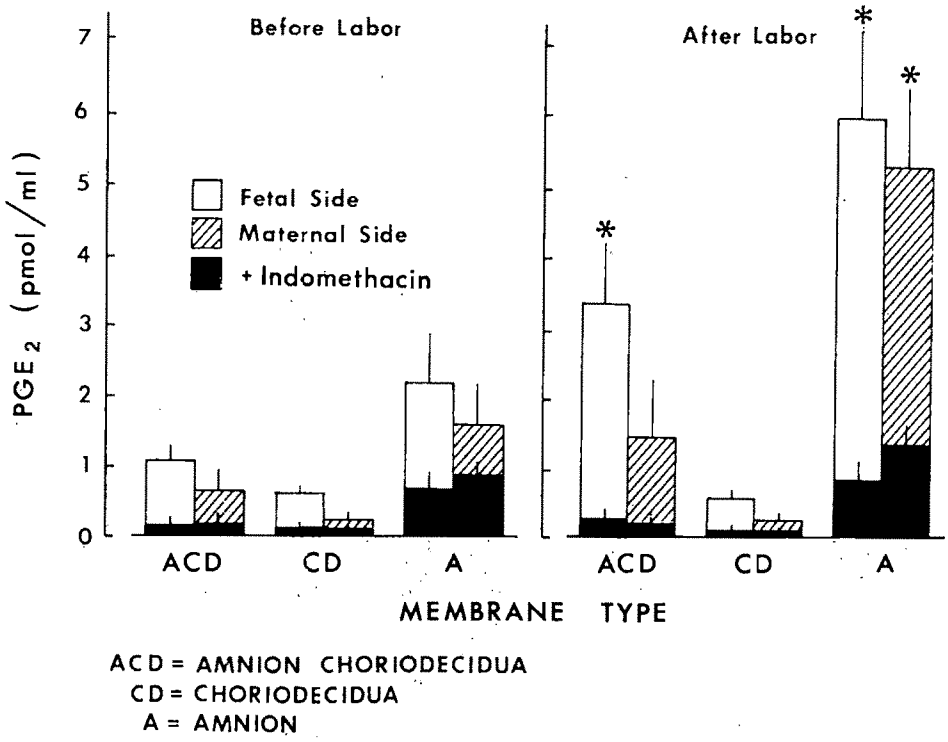


Fig. 1. PGE_2 concentrations (mean ± SEM) on the fetal *(open bars)* and maternal *(hatched bars)* sides of membranes. *ACD,* amnion chorion-decidua (intact); *CD,* chorion-decidua; and *A,* amnion. Incubations were for 4 hours in medium alone or with addition of 1 μmol/L indomethacin *(solid bars),* with membranes from before ($n = 10$) and after ($n = 9$) labor. *Asterisks* denote a significant increase in PGE_2 after labor over that of matched membrane type and side from before labor for net production (see text).

unpaired two-tailed t tests, simple and multiple regression, and correlation analyses.

Results

Net PGE_2 production by control membranes was calculated by subtraction of PGE_2 elution in the presence of indomethacin from PGE_2 production in incubation medium alone. The results are summarized in Fig. 1. A comparison of each membrane type by general linear model analysis of variance of membrane side and condition of labor identified a significant increase in net postlabor PGE_2 production by the fetal side of intact membrane ($F = 5.72; p = 0.02$) and both fetal and maternal sides of postlabor amnion ($F = 9.72; p = 0.003$). Chorion-decidua displayed no change in PGE_2 production between prelabor and postlabor conditions on either the fetal or maternal sides.

The addition of PGE_2 at 0 hours to the fetal side of each membrane type incubated in the presence of indomethacin resulted in a progressive decline in PGE_2 on the fetal side of all membranes obtained from both prelabor and postlabor deliveries (Fig. 2). However, a concomitant increase in PGE_2 on the maternal side was observed only when amnion was used (Fig. 2, *C*). No change in PGE_2 concentrations over baseline levels was detected on the maternal sides of the intact membranes or chorion-decidua preparations (Fig. 2, *A* and *B*). The amounts of PGE_2 recovered at 4 hours on the fetal and maternal sides of each membrane preparation from the above experiments were compared with the concentration of PGE_2 added to the fetal chamber at 0 time (Table II). When the individual membrane types were compared in the amount, percentage or both of recoverable PGE_2 between prelabor and postlabor conditions, no significant difference was observed. However, comparison between membrane preparations within each condition of labor revealed highly significant ($p < 0.001$) differences in recoverable PGE_2. The data from amnion indicated that 24% to 26% of PGE_2 is transported from the fetal to maternal side over 4 hours. However, it was not evident from these experiments that PGE_2 was translocated by chorionic membrane. From the decline in PGE_2 on the fetal side of chorion-decidua and intact membrane and failure of PGE_2 appearance on the maternal side, it can be computed that after 4 hours, chorionic metabolism of PGE_2 accounts for between 33% and 56% of the exogenously added prostanoid to PGE_2 metabolites. Furthermore, addition of a bolus of PGE_2 to the fetal side of intact membrane at time 0 and incubated without any prostaglandin synthetase inhibitor present resulted in PGE_2 concentrations on the maternal side after 4 hours of 0.41 ± 0.18 pmol/ml before

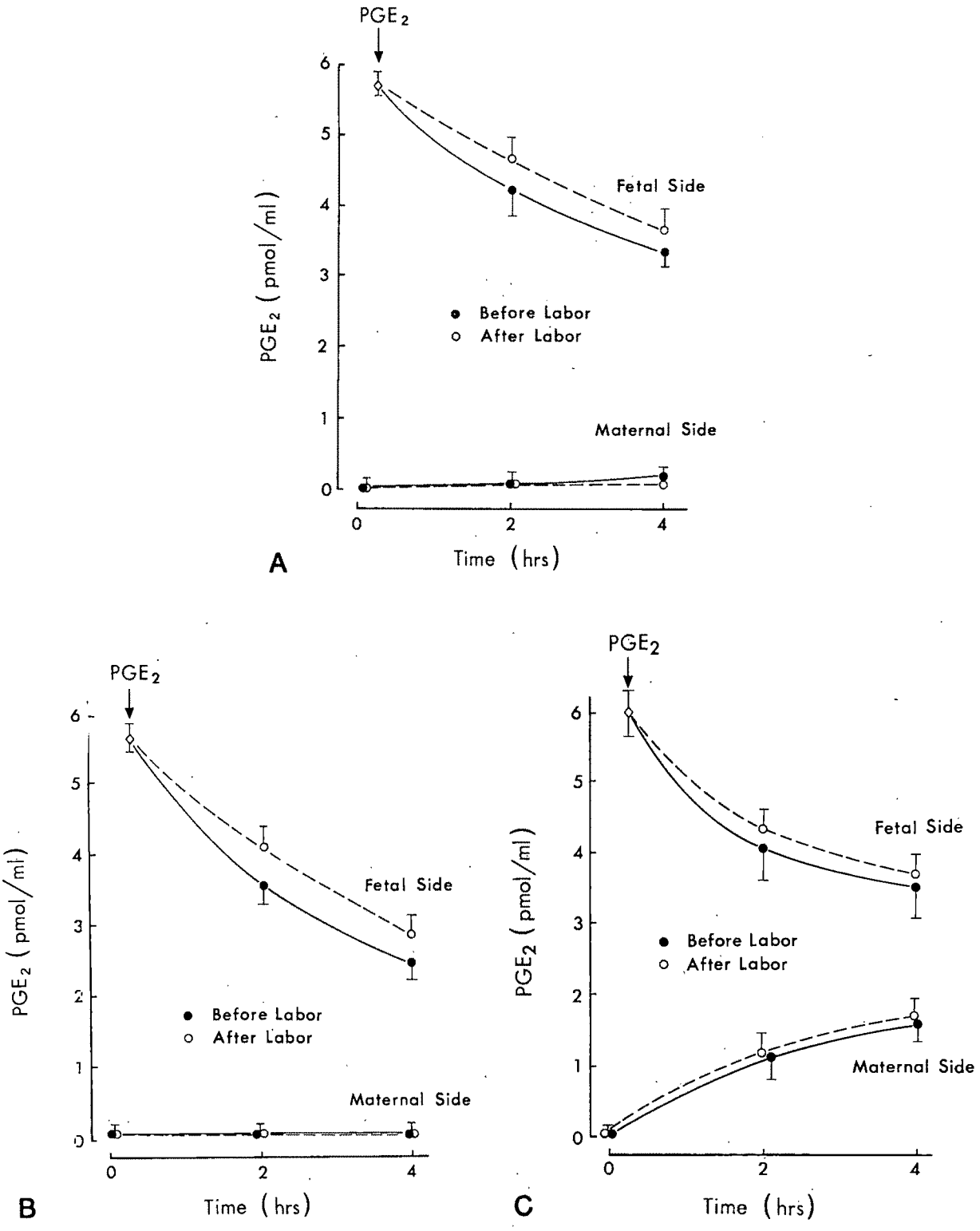


Fig. 2. Translocation of PGE_2 across amnion chorion-decidua (intact) **(A)**, chorion-decidua **(B)**, and amnion **(C)** after indomethacin (1 μmol/L) treatment and bolus PGE_2 (6 nmol/L). Membranes were from before labor (n = 10) and after labor (n = 9). Values are mean ± SEM.

labor and 1.16 ± 0.56 pmol/ml after labor (mean ± SEM); the values were not significantly different. Similarly, the PGE_2 concentrations on the maternal side of control intact membranes (no PGE_2 or indomethacin added) before labor were 0.51 ± 0.27 pmol/ml and 1.21 ± 0.54 pmol/ml after labor. The same experiment with chorion-decidua resulted in no change in PGE_2 concentrations on the maternal side over those of

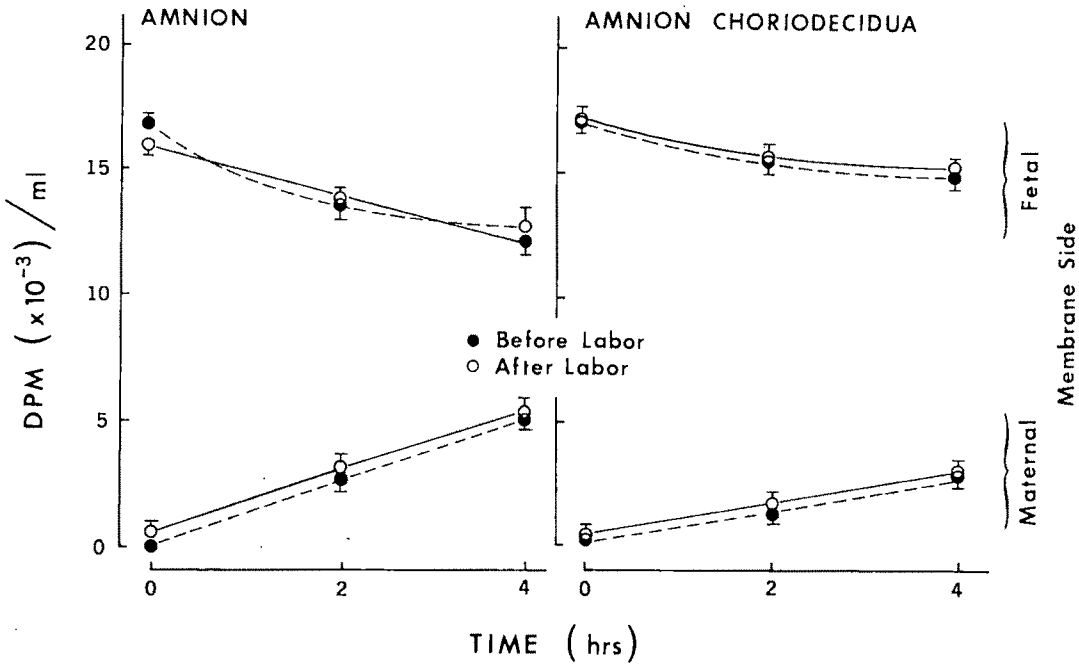


Fig. 3. Translocation of radiolabeled PGE_2 and metabolites across amnion and amnion-chorion-decidua from before ($n = 5$) and after ($n = 5$) labor delivery. [^{3}H]PGE_2 was added at time 0 to fetal chamber of perfusion apparatus. Values represent means ± SEM.

Table II. Comparison of amount of PGE_2 added to fetal side of membranes in presence of indomethacin at 0 hours with that recovered by RIA analysis on fetal and maternal sides at 4 hours of incubation

	PGE_2 (pmol/ml) (mean ± SEM)					
	Prelabor			*Postlabor*		
Membrane	*Added*	*Recovered*	*%**	*Added*	*Recovered*	*%**
Intact (total)	5.74 ± 0.17			5.69 ± 0.24		
Fetal chamber		3.51 ± 0.21	61		3.75 ± 0.39	66
Maternal chamber		0.11 ± 0.03	2		0.05 ± 0.03	1
TOTAL		3.61 ± 0.32	63		3.80 ± 0.43	67
Chorion-decidua	5.70 ± 0.16			5.71 ± 0.28		
Fetal chamber		2.45 ± 0.15	43		2.91 ± 0.32	51
Maternal chamber		0.05 ± 0.03	1		0.07 ± 0.05	1
TOTAL		2.50 ± 0.34	44		2.98 ± 0.35	52
Amnion	6.08 ± 0.2			5.91 ± 0.49		
Fetal chamber		3.76 ± 0.39	62		3.71 ± 0.48	63
Maternal chamber		1.46 ± 0.25	24†		1.55 ± 0.10	26†
TOTAL		5.22 ± 0.25	86		5.26 ± 0.11	89

*%, Percent of total added at 0 hours.

†Significantly different from intact and chorion-decidua maternal side values, $p < 0.001$.

chorion-decidua controls (data not shown). Thus, despite maintenance of high PGE_2 levels on the fetal side of intact and chorion-decidua membranes, we found no evidence of PGE_2 translocation to maternal side.

To further characterize PGE_2 metabolism, [^{3}H]PGE_2 was added to the amnion and intact membrane preparations, and the metabolic products in the fetal and maternal compartments were identified by HPLC (Fig. 3). When amnion was used and [^{3}H]PGE_2 was placed on the fetal side, approximately 30% of added radioactivity was translocated to the maternal side after 4 hours. When the intact membrane preparation was used, approximately 15% of tritium translocated to the maternal chamber by 4 hours. There was no significant difference in translocation or acccumulation of tritium in membranes relative to labor status. Results of HPLC

Table III. Percent distribution of tritiated prostaglandins on fetal and maternal sides of amnion after 4 hours' exposure to [^{3}H]PGE_2 on fetal side (mean ± SEM)*

	Before labor (n = 5)				*After labor (n = 5)*			
	F	%	*M*	%	*F*	%	*M*	%
PGE_2	95.0 ± 1.5	65	95.5 ± 2.1	25	95.2 ± 1.4	65	95.3 ± 1.5	27
PGE_2-M†	0.3 ± 0.1	1	0.7 ± 0.2	1	0.3 ± 0.2	0.5	0.1 ± 0.0	0
15K-PGE_2	1.3 ± 0.4	1	0.7 ± 0.5	0.5	0.2 ± 0.1	0.5	0.9 ± 0.4	1
15K-H_2-PGE_2	3.0 ± 1.8	2	0.2 ± 0.1	0.5	0.7 ± 0.3	1	0.9 ± 0.8	1
Bicyclic prostaglandins‡	1.5 ± 0.4	1	1.5 ± 0.6	1	1.3 ± 0.4	1	1.8 ± 0.5	1
Unidentified§	0.7 ± 0.8‖	1	1.5 ± 0.5	1	1.1 ± 0.4	2	1.3 ± 0.5	1
Total dpm (%)	111,600	71	47,700	29	118,800	70	52,200	31

%, Percent of total [^{3}H]PGE_2 added at time 0 to chamber on fetal side of amnion. 100%, Both fetal and maternal chambers.
*Average of five replicates.
†Tentatively identified as ω and β-oxidation products of PGE_2.
‡11-Deoxy-13,14-dihydro-15-keto-11β,16ε-cyclo-PGE_2.
§Excludes thromboxane B_2, $PGF_{2\alpha}$, PGD_2, 15-keto-H_2-$PGF_{2\alpha}$.
‖Eluted beyond bicyclic prostaglandin.

Table IV. Percent distribution of tritiated prostaglandins on fetal and maternal sides of intact membrane after 4 hours' exposure to [^{3}H]PGE_2 on fetal side (mean ± SEM)*

	Before labor (n = 5)				*After labor (n = 5)*			
	F	%	*M*	%	*F*	%	*M*	%
PGE_2	75.7 ± 5.4	55	0	0	72.5 ± 0.4	57	0	0
PGE_2-M†	3.3 ± 0.7	2	21.3 ± 23.2	2	0.4 ± 0.3	1	14.2 ± 3.9	1
15K-PGE_2	0.5 ± 0.3	1	4.0 ± 2.7	1	0.3 ± 0.3	1	16.9 ± 5.3	1
15K-H_2-PGE_2	9.6 ± 2.3	8	52.1 ± 15.7	3	13.1 ± 2.9	12	40.3 ± 13.5	2
Bicyclic prostaglandins‡	3.6 ± 1.7	1	22.7 ± 14.1	2	2.6 ± 1.2	2	34.1 ± 5.4	2
Unidentified§	5.9 ± 1.5	5	0	0	11.0 ± 1.4‖	10	4.5 ± 1.8	0
Total dpm* (%)	135,000	72	24,300	8	139,500	83	26,100	7

%, Percent of total [^{3}H]PGE_2 added at time 0 to chamber on fetal side of intact membrane.
*Average of five replicates.
†Tentatively identified as ω- and β-oxidation products of PGE_2.
‡11-Deoxy-13,14-dihydro-15-keto-11β,16ε-cyclo-PGE_2.
§Excludes thromboxane B_2, $PGF_{2\alpha}$, PGD_2, 15-keto-H_2-$PGF_{2\alpha}$.
‖Eluted beyond bicyclic prostaglandins.

analysis of tritiated compounds in fluids from the fetal and maternal chambers of amnion and intact membrane are summarized in Tables III and IV. Approximately 25% of the radioactivity was found in the maternal chamber after a 4-hour incubation with the before-labor amnion and 27% with the after-labor amnion. This radioactivity comigrated with authentic PGE_2 on the HPLC. This value is similar to the PGE recovery by RIA analysis (Table III). The analyses of intact membrane incubates failed to identify any [^{3}H]PGE_2 on the maternal side, whereas 55% to 57% of compound on the fetal side was [^{3}H]PGE_2. Tritium recovery from the HPLC columns was 96.3% and 82.6% for amnion and intact membrane, respectively. The major metabolite in the maternal chamber of intact membranes was 15-keto-13,14-dihydro-PGE_2 both before and after labor; concentrations were 52.1% and 40.3%, respectively. Additional PGE_2 metabolites coeluted with authentic 15-keto-PGE_2 and the bicyclic derivative of the 15-keto-13,14-dihydro-PGE_2 (Fig. 4). However, no radiolabeled PGE_2 could be detected on the maternal side when the intact membrane preparation was used. Less than 0.5% of the added total radioactivity was present in the tissue after the 4-hour incubation. Therefore the radioactivity of the tissues was not further fractionated into the various metabolites of PGE_2.

Comment

On the basis of reported findings, it is suggested that PGE_2 can be translocated across isolated amnion. However, the translocation of exogenously added PGE_2 across the chorion-decidua appears to be impeded by enzymatic metabolism of PGE_2 in this tissue. Earlier

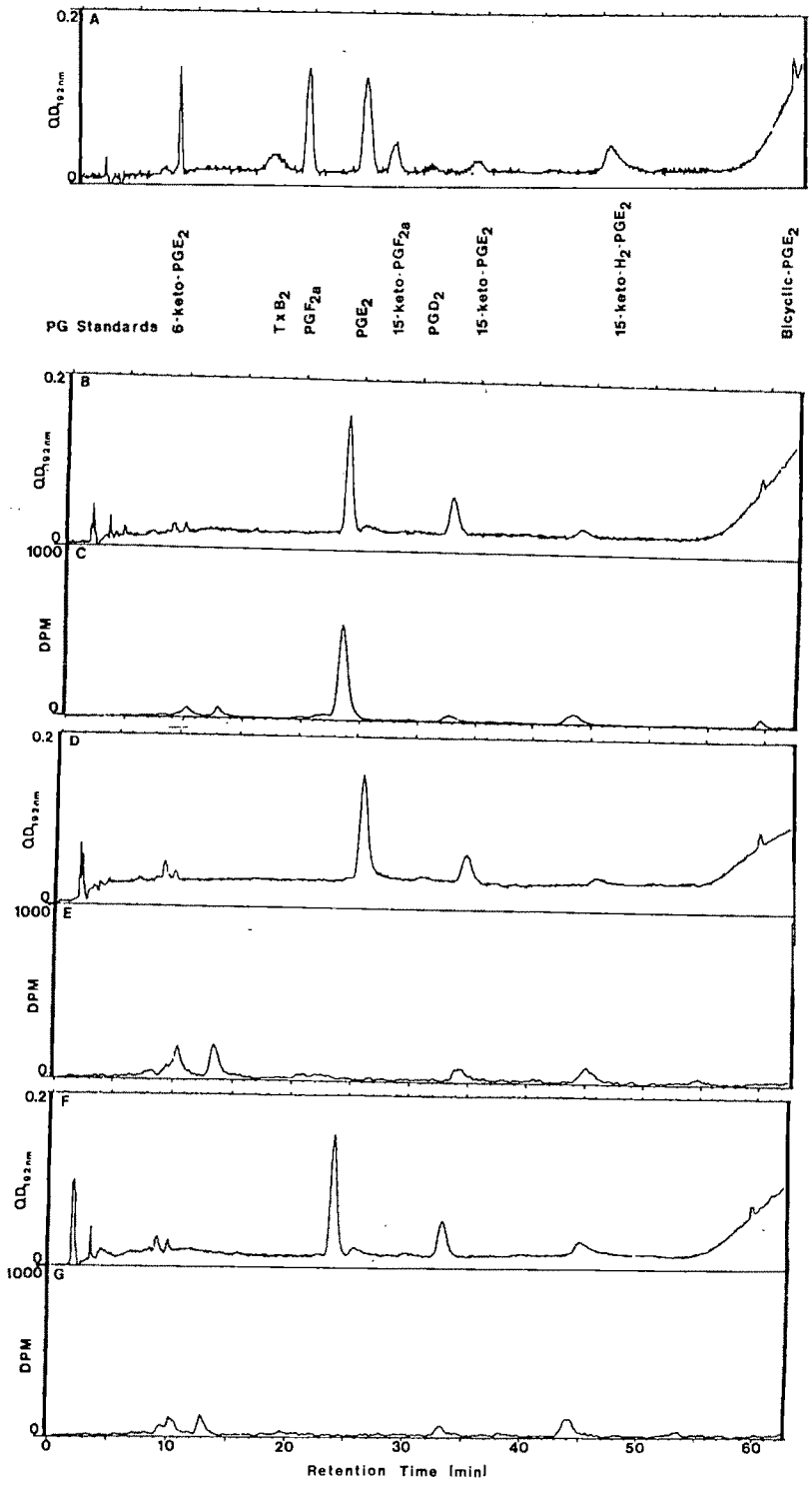


Fig. 4. Representative HPLC fractionations of prostaglandins *(A, B, D, F)* detected at 192 nm and associated radioactivity *(C, E, G)* after [^{3}H]PGE_2 bolus to fetal side of membranes. *A*, Separation of authentic prostaglandin standards; tritium in fetal *(B, C)* and maternal *(D, E)* chambers at 4 hours of "before labor" intact membrane preparation; tritium in maternal chamber at 4 hours of "after labor" intact membrane preparation *(F, G)*. Carrier PGE_2, 15-keto-PGE_2, and 15-keto-H_2-PGE_2 were added to all samples (see *B, D,* and *F*) for identification of metabolites.

studies identified the capacity of chorion laeve to metabolize PGE_2.[1,2] Amnion tissue has a high capacity for PGE_2 synthesis and a limited capacity to inactivate PGE_2 because of the absence of 15-hydroxyprostaglandin dehydrogenase activity.[1,2] We and others[1,2] have observed that chorion laeve and decidua vera tissues also have the capacity for the synthesis of both PGE_2 and $PGF_{2\alpha}$.[1,2] In addition, these two latter tissues have a

significant amount of prostaglandin dehydrogenase activity.[1, 2] The present observations on the translocation of PGE_2 are consistent with these enzymatic studies in that both endogenously synthesized and the exogenously added PGE_2 were translocated across the amnion membrane unchanged, whereas PGE_2 was metabolized completely when intact membranes were used. Recently Nakla et al.[6] and Bennett et al.[12] suggested that most amniotic PGE_2 translocates unmetabolized across amnion-chorion plus decidua. However, our results confirm earlier observations that PGE_2 concentrations do not increase on the maternal side of isolated chorion-decidua or intact membrane despite increased amniotic output after labor.[7] Furthermore, our HPLC analysis failed to identify any unmetabolized [^{3}H]PGE_2 on the maternal side of intact membrane regardless of labor status, and RIA of PGE_2 also failed to detect any increase on the maternal side despite a substantial loss of added PGE_2 on the fetal side. Each membrane preparation used in our study was confirmed to be leak-free, and the incubation medium we used is known to maintain cellular integrity of the fetal membranes.[8] Furthermore, Fig. 3 clearly identifies translocation of tritiated compound across the membranes, confirming that there was no blockage of diffusion in our system.[7, 9] More recently, Collins and Roberts[13] also failed to identify transfer of amniotic fluid PGE_2 from the fetal to maternal side of intact fetal membrane despite increased PGE_2 synthesis in amnion. The disparate results between the various groups with regard to the PGE_2 translocation across the fetal membranes is not readily apparent. However, on the basis of our data, it is suggested that amnion is not the source of PGE_2 on the myometrial side of reflected membrane during labor. It should be emphasized that these findings do not discount the potential for activation of amnion by prostanoids or other autacoids which, in turn, activates the adjacent chorion in labor-related metabolic events.

We have suggested that a second autacoid, namely platelet-activating factor, may indeed function by such a mechanism.[14] Platelet-activating factor has been reported to activate glycogen breakdown in the perfused rat liver.[15] Furthermore, platelet-activating factor levels in term amnion before labor are equivalent to amnion from early gestation (14 to 16 weeks) but twofold greater in postlabor amnion. In addition there is an in vitro platelet-activating factor–induced efflux of PGE_2 by term amnion[14] and amnion cells in culture.[16] Similar to PGE_2, platelet-activating factor concentrations in amniotic fluid are markedly elevated during labor at term[14] and in both preterm labor and premature rupture of membranes.[17] Recently we reported that the wet weight of reflected amnion increased with labor and that this may result from the hydrolysis of the thick underlying collagen layer in preparation for membrane rupture and disociation from the uterus at parturition.[9] In addition, prostaglandin-mediated collagenase activity is reported to occur in the follicular wall before its rupture at time of ovulation, and cervical effacement caused by collagen breakdown can be induced by PGE_2.[18] Thus increased amnionic PGE_2 synthesis may serve other currently undefined functions in labor and parturition.

Failure of amniotic PGE_2 to reach the myometrial surface of the reflected membranes does not negate the presence of the prostanoid in such tissue. The control experiments clearly identify PGE_2 production on the decidual surface of the membranes. However, we were unable to identify any significant increase in PGE_2 production on the maternal surface despite a substantial increase in amniotic productivity after labor. This is similar to our previous observations.[7] Thus we suggest that the availability of PGE_2 per se on the maternal surface of the membranes is steady during labor and independent of PGE_2 productivity by amnion.

Because both decidua and myometrium produce PGE_2,[1, 2] availability of PGE_2 in the myometrium may be exclusively maternal in origin. Furthermore, a role for PGE_2 in contractility may be at the level of prostaglandin receptors on myometrial cells. However, myometrial receptors for PGE series and $PGF_{2\alpha}$ appear quantitatively equivalent before and after labor.[19] Furthermore, myometrium converts PGE_2 to $PGF_{2\alpha}$, and to 13,14-dihydro-15-oxo metabolites on both prostaglandins at an equivalent rate before and after labor.[20] Thus PGE_2 production and metabolism by chorion, decidua, and myometrium appear to be unaffected by labor. Conversely, $PGF_{2\alpha}$ production by the decidua[21] and the decidual surface of intact fetal membrane[22] is reported to increase with labor. In maternal circulation, the concentration of PGF metabolites rises substantially during labor, whereas PGE metabolite concentrations are not altered.[1] Because the rates of production of PGE_2 by intact fetal membrane increase with labor only on the fetal side and that of $PGF_{2\alpha}$ only on the maternal side, respectively, we suggest that dynamic changes in the ratio of PGE_2 to $PGF_{2\alpha}$ by each of amnion, chorion, and probably decidua are important to the process of labor. A change in prostaglandin ratios may impart a synergistic effect that positively influences myometrial contraction. Other prostanoids and autacoids such as platelet-activating factor, probably play an important role in maintaining the contractions of labor.[14]

In summary, the results of these experiments suggest that PGE_2 of amnionic origin is unavailable to the myometrium because of its metabolism by the underlying chorion. Thus PGE_2 alone may not be the sole initiating factor of labor because its availability on the maternal surface appears not to change between prelabor and postlabor conditions. Thus we suggest that a fundamental process such as labor is more likely the result of multiple factors rather than the so-called "trigger" hypothesis involving a single compound, as invoked by some investigators.

We greatly appreciate the expert technical assistance of Ms. Collette Odowichuk, Ron White, and Catherine Robinson during these experiments.

REFERENCES

1. Keirse MJNC. Eicosanoids in human pregnancy and parturition. In: Mitchell MD, ed. Eicosanoids in reproduction. Boca Raton, Florida: CRC Press, 1990:199.
2. Bleasdale JE, Johnston JM. Prostaglandins and human parturition: regulation of arachidonic acid metabolism. Rev Perinatal Med 1984;5:151.
3. Neider J, Augustin W. Increase in prostaglandin E and F equivalents in amniotic fluid during late pregnancy and rapid PGF elevation after cervical dilatation. Prostaglandins Leukotrienes Med 1983;12:289.
4. Schwarz BE, Schultz FM, MacDonald PC, Johnston JM. Initiation of human parturition. III. Fetal membrane content of prostaglandin E_2 and $F_{2\alpha}$ precursor. Obstet Gynecol 1975;46:564.
5. Mitchell MD, Bibby J, Hicks BR, et al. Specific production of prostaglandin E_2 by tissues from human uterus and fetoplacental unit. Prostaglandins 1978;15:377.
6. Nakla S, Skinner K, Mitchell BF, et al. Changes in prostaglandin transfer across human fetal membranes after spontaneous labor. AM J OBSTET GYNECOL 1986;155: 1337.
7. McCoshen JA, Johnson K, Dubin H, et al. Prostaglandin E_2 release on the fetal and maternal sides of the amnion and chorion-decidua before and after term labor. AM J OBSTET GYNECOL 1987;156:173.
8. Schwartz AL, Forster CS, Smith P, et al. Human amniotic metabolism. I. In vitro maintenance. AM J OBSTET GYNECOL 1977;127:470.
9. McCoshen JA, Tullock HV, Johnson KA. Umbilical cord is the major source of prostaglandin E_2 in the gestational sac during term labor. AM J OBSTET GYNECOL 1989;4:973.
10. Dubin NH, Ghodgaonkar RB, King TM. Role of prostaglandin production in spontaneous and oxytocin-induced uterine contractile activity in in vitro pregnant rat uteri. Endocrinology 1979;105:47.
11. Campbell WB, Ojeda SR. Measurement of prostaglandins by radioimmunoassay. Methods Enzymol 1987;141:323.
12. Bennett PR, Chamberlain GVP, Patel L, et al. Mechanisms of parturition: the transfer of prostaglandin E_2 and 5-hydroxyeicosatetraenoic acid across fetal membranes. AM J OBSTET GYNECOL 1990;162:583.
13 Collins PL, Roberts JM. Prostaglandin signal transduction through fetal membranes in human parturition [Abstract 198]. In: Proceedings of the thirty-seventh annual meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
14. Johnston JM, Maki N, Angle MJ, Hoffman DR. Regulation of the arachidonic acid cascade and PAF metabolism in reproductive tissues. In: Mitchell MD, ed. Eicosanoids in reproduction. Boca Raton, Florida: CRC Press, 1990;5.
15. Hanahan DJ. Platelet activating factor: a biologically active phosphoglyceride. Ann Rev Biochem 1986;55:483.
16. Bleasdale JE, Bala GA, Thakur NR, et al. Regulation of prostaglandin production by intrauterine tissue. In: Cosmi E, DiRenzo, eds. Proceedings of the European congress of perinatal medicine. 1989:828.
17. Hoffman DR, Romero R, Johnston JM. Detection of platelet-activating factor in amniotic fluid of complicated pregnancies. AM J OBSTET GYNECOL 1990;162:525.
18. Lipner H. Mechanism of mammalian ovulation. In: Knobil E, Neill JD, eds. The physiology of reproduction. vol 1. New York: Raven Press, 1988:447.
19. Giannopoulis G, Jackson K, Kredenster J, et al. Prostaglandin E and $F_{2\alpha}$ receptors in human myometrium during the menstrual cycle and in pregnancy and labor. AM J OBSTET GYNECOL 1985;153:904.
20. Solar RC, Bernal AL, Turnbull AC. Prostaglandin E_2 metabolism by human myometrium. J Clin Endocrinol Metab 1988;2:402.
21. Skinner KA, Challis JRG. Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor. AM J OBSTET GYNECOL 1985;151: 519.
22. Kredenster JV, McCoshen JA, Odowichuk C, et al. Prostaglandin $F_{2\alpha}$ is the predominant prostaglandin available to the myometrial side of human membranes and is not influenced by high prostaglandin E_2 concentrations on the fetal surface [Abstract 65]. In: Proceedings of the forty-fourth annual meeting of the Society of Obstetricians and Gynaecologists of Canada, Vancouver, British Columbia, Canada, June 22-25, 1988.

Surgical staples in cesarean section: A randomized controlled trial

Marc G. Villeneuve, MD, Samir Khalifé, MD, Sylvie Marcoux, MD, PhD, and Pierre Blanchet, MD

Sainte-Foy, Quebec, Canada

This randomized controlled trial compares the use of the Auto Suture Poly CS 57 disposable surgical stapler (n = 98) with standard hysterotomy (n = 102) in low transverse cesarean sections. Subjective assessment of blood loss by the surgeon resulted in lower mean (±SEM) total blood loss estimates in the stapled group (492 ± 24 ml) than in the nonstapled group (579 ± 38 ml) (p = 0.05). However, surgeon's estimation of blood loss as a result of the hysterotomy and blood loss estimated by the hemoglobin deficit measured on the second postoperative day did not significantly differ between the two groups. The use of the stapling device tended to lengthen the total operating time, which averaged 42.5 minutes in the group with the staples and 39.2 minutes in the group with the standard hysterotomy (p = 0.05). The risk of febrile morbidity, the frequency of endometritis, and the length of hospitalization were similar in the two groups. Our results do not support the routine use of the Auto Suture Poly CS 57 disposable surgical stapler in low transverse cesarean sections. (Am J Obstet Gynecol 1990;163:1641-6.)

Key words: Absorbable staples, cesarean section

The Auto Suture Company has marketed the Auto Suture Poly CS* 57 disposable surgical stapler with a disposable loading unit of copolymer Lactomer* absorbable staples. The loading units cost approximately $200 per patient. This device has gained acceptance in many surgical specialties. Unlike the original stapling device with metal staples,[1, 2] the use of the Lactomer absorbable surgical staples for hysterotomy has been shown not to impair subsequent fertility in nonhuman primates.[3] It has therefore been suggested that this stapling device could be used as an alternative to the standard hysterotomy procedure in human cesarean section.

The Auto Suture Poly CS 57 disposable surgical stapler could offer advantages over the standard hysterotomy procedure. First, the stapling device was designed to reduce the amount of bleeding caused by the hysterotomy and hence should lower the risk of transfusion-related complications. Second, the inert copolymer staples do not compromise the microcirculation of the uterine wall as much as the absorbable sutures do, leading to less inflammation and less ischemia in the uterine healing process. These two characteristics should diminish the risk of infection and favor a more resistant hysterotomy healing for subsequent vaginal deliveries. The less inflammation in the vesicouterine space, the less adherent will be the bladder to the lower uterine segment in the event of a repeat cesarean section. Furthermore, if the Auto Suture Poly CS 57 disposable surgical stapler could reduce the frequency of endometritis after a cesarean section, as recently suggested by Burkett et al.,[4] then hospital stay and costs could be lowered too. Finally, by reducing bleeding the Auto Suture Poly CS 57 disposable surgical stapler could shorten operating time.

To our knowledge, only one previous study has evaluated the use of surgical staples for cesarean section in human beings,[4] and it was not a randomized controlled trial. The purpose of our study was to assess whether performance of a low transverse hysterotomy with the Auto Suture Poly CS 57 disposable surgical stapler in cesarean section, in comparison with a standard hysterotomy procedure, reduces blood loss, operating time, febrile morbidity, and length of postoperative hospitalization.

Material and methods

The study was carried out at Saint-Sacrement Hospital, a hospital affiliated with Laval University in Quebec City, Quebec, Canada. All patients who needed a cesarean section from Nov. 18, 1987, to April 14, 1989, were eligible for the study. Patients who agreed to par-

From the Department of Obstetrics and Gynecology and the Department of Social and Preventive Medicine, Laval University.
Supported by Auto Suture Canada Ltd.
Received for publication February 6, 1990; revised July 12, 1990; accepted July 20, 1990.
Reprint requests: Dr. Sylvie Marcoux, Département de Médecine Sociale et Préventive, Faculté de Médecine, Université Laval Sainte-Foy, Quebec, Canada G1K 7P4.
**Reg. U.S. Patent and Trademark Office. Auto Suture Company, Division of United States Surgical Corporation, Norwalk, Conn.*
6/1/24034

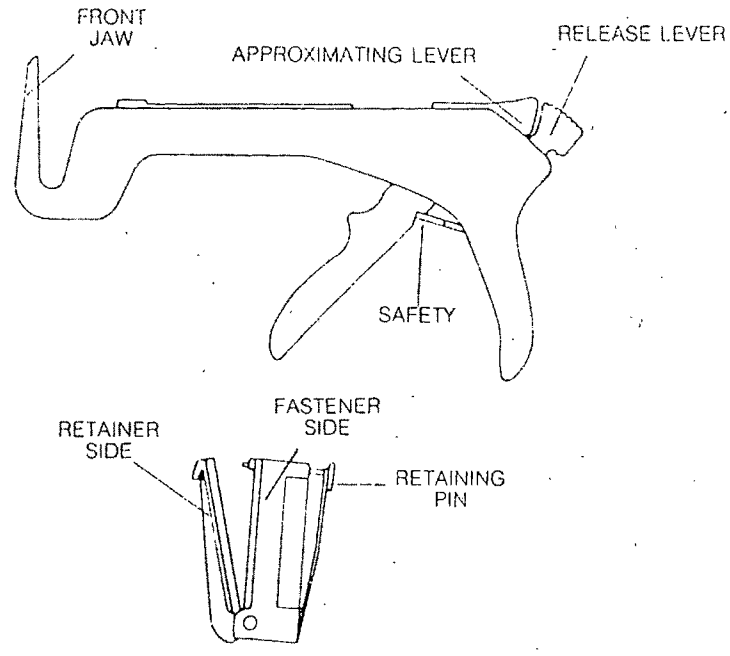


Fig. 1. Schematic view and nomenclature of the Auto Suture Poly CS 57 disposable surgical stapler and Auto Suture Poly CS 57 disposable surgical stapler loading unit.

ticipate signed an informed consent form. The study was approved by the hospital's ethics committee. Women enrolled in the study were randomly allocated to one of two hysterotomy techniques: hysterotomy with the Auto Suture Poly CS 57 disposable surgical stapler or standard hysterotomy. The allocation scheme was generated with a table of random numbers and the randomization was achieved with a series of sealed numbered envelopes.

All cesarean sections were performed by the attending physician and a resident. The whole procedure was the same for the two groups except for the hysterotomy technique. In the group with the standard technique, the lower uterine segment was incised with a scalpel on a distance of 2 cm after the bladder was reclined downward. The incision was extended bilaterally in a transverse fashion with bandage scissors or by digital traction. In the group with the stapler, a 2-cm hysterotomy incision was made with a scalpel in the lower uterine segment. The stapling device (Fig. 1) was then introduced between the membranes and the uterine wall with its distal end pointed laterally and slightly upward. The stapler was then fired, which caused two columns of eight absorbable staples each to be applied parallel to the lower uterine segment. As the staples were closed, a cutting device was then activated and the hysterotomy was performed between the two rows of staples. The same procedure was repeated on the other side. The membranes were ruptured and the infant was delivered. Extreme caution was taken not to include any fetal parts or umbilical cord in the stapling device. Once the placenta was delivered, the hysterotomy was closed with a single layer of absorbable chromic catgut suture 0, taking care to symmetrically appose the two rows of staples. This continuous nonlocked suture was passed through the middle of each staple starting at the lateral extremity on each side of the hysterotomy and ending at the center.

Antibiotic prophylaxis was given to all patients who had been in labor for >6 hours or had their membranes ruptured for >6 hours. Prophylaxis consisted of intravenous administration of cefazolin 1 gm or cefoxitin 2 gm at the time the umbilical cord was clamped. During the procedure the nursing staff systematically recorded the operating time. Different time spans were calculated: time from abdominal wall incision to birth, time from opening of the uterus to uterine closure, and total operating time from abdominal wall incision to abdominal wall closure.

Immediately after the cesarean section the surgeon recorded his/her subjective assessment of total blood loss and his/her assessment of blood loss attributed to the hysterotomy. Technical difficulties or complications related to the use of the stapling device were also noted.

The coagulation parameters (prothrombin time, partial thromboplastin time, and bleeding time) and the hemoglobin value were obtained for all patients before the cesarean section. The difference between the hemoglobin concentration at admission and the hemoglobin concentration on the second postoperative day

Table I. Characteristics of the patients in the two study groups

Characteristics	*Hysterotomy with stapling device (n = 98)*	*Standard hysterotomy (n = 102)*	*p Value*
Age (yr)*	29.4 ± 0.4	29.5 ± 0.4	0.82
Primiparous patients (%)	33.7	45.1	0.13
Admission weight (kg)*	75.6 ± 1.4	73.6 ± 1.2	0.27
Multiple gestations (%)	5.1	4.9	1.00
Labor before cesarean section (%)	74.5	68.6	0.45
Intact membranes at cesarean section (%)	78.6	68.6	0.15
Peridural anesthesia (%)	95.9	93.1	0.58
Antibioprophylaxis (%)	18.4	24.5	0.38
Abnormal coagulation tests (%)	5.1	5.9	1.00
Primary cesarean section (%)	42.9	56.9	0.07
Tubal ligations (%)	25.5	21.6	0.62
Birth weight ≥4000 gm (%)†	14.0	11.3	0.74
Birth weight (gm)*†	3498 ± 49	3429 ± 46	0.31

*Mean ± SEM.
†Among singletons.

was calculated for each patient. Medical charts were reviewed by one of us (M. V.) to establish the presence of postoperative febrile morbidity according to Monif's criteria,[5] document the presence of immediate postoperative morbidity, and estimate the length of postoperative hospitalization.

Statistical differences between the two study groups were assessed by the Student *t* test for means and by the χ^2 or Fisher exact tests for proportions.

Results

Of the 386 patients who had a cesarean section during the study period, 200 were included in the trial. Among the 186 nonparticipants, 43 (23.1%) refused to participate, 41 (22.0%) needed an emergency cesarean section and could not have the protocol explained to them, 91 (48.9%) were not approached because their obstetrician either omitted the explanation (78) or refused to explain the protocol (13), and 11 (5.9%) were excluded because of other miscellaneous reasons. The proportions of repeat cesarean sections and of elective interventions were higher among participants (50.5% and 33.5%, respectively) than the proportions among nonparticipants (25.8% and 25.3%, respectively).

Among the 200 enrolled subjects, 102 were randomized to the standard technique and 98 to the stapling device. Ten subjects had a standard hysterotomy albeit they were allocated to the stapler group. Of these, two belonged to the first 100 enrolled subjects and eight to the second 100 patients. The reasons for protocol deviation were as follows. For two patients, the surgeon forgot about the stapling device and extended the 2 cm hysterotomy with his fingers. In three women, two of whom had a prolonged bleeding time, the stapling device could not be inserted because of substantial bleeding from the small hysterotomy site. Two other patients had a spontaneous uterine dehiscence after the 2 cm scalpel hysterotomy. Three cases were considered technically too difficult (e.g., overweight patient with two previous cesarean sections).

Finally, two patients from the stapler group had a hysterotomy with staples combined with a scalpel or manual extension of hysterotomy. In one of these patients, an inverted "T" incision was needed to deliver a fetus in transverse lie; in the other patient, the obstetrician abandoned the stapler because the stapler insertion on the first side caused a uterine tear and active bleeding.

Patient characteristics in the two study groups. Patients were considered to be in the group to which they had been allocated, whether or not the stapling device was used. Baseline characteristics of the study groups are shown in Table I. Nine obstetricians participated in the study. The distribution of patients according to the obstetrician involved in the procedure was similar in the two groups. Except for a lower proportion of primary cesarean sections in the group with the stapling device, the two groups harbored only small differences with regard to the factors that could influence the study outcomes. Eight patients in the stapling device group (8.2%) and 12 (11.8%) in the standard hysterotomy group had more than one previous cesarean section (χ^2 = 0.37, 1 degree of freedom[df], p = 0.54). All previous incisions were low transverse. Besides tubal ligations, no additional interventions were performed in either group. The proportion of patients who were in labor or had their membranes ruptured before the cesarean section did not differ between the two groups. Among patients in labor, the mean duration of labor was similar in both groups: 9.9 (SEM, 1.2) hours in the stapler group versus 10.1 (SEM, 0.9) hours in the standard technique group (t = 0.10, 55 degrees of free-

Table II. Comparison of blood loss and hemoglobin deficit between the two study groups

Blood loss and cesarean type	*Hysterotomy with stapling device*			*Standard hysterotomy*			*p Value*
	No.	*Mean ± SEM*	*Range*	*No.*	*Mean ± SEM*	*Range*	
Total blood loss (ml)							
Primary cesarean section	42	510 ± 38	100-1100	58	572 ± 39	100-1500	0.27
Repeat cesarean section	56	479 ± 31	100-1000	44	587 ± 71	80-2500	0.17
TOTAL	98	492 ± 24	100-1100	102	579 ± 38	80-2500	0.05
Blood loss due to hysterotomy (ml)							
Primary cesarean section	42	239 ± 31	0-900	57*	291 ± 39	50-1500	0.30
Repeat cesarean section	56	241 ± 26	0-900	44	297 ± 47	20-1500	0.30
TOTAL	98	240 ± 20	0-900	101*	294 ± 30	20-1500	0.14
Hemoglobin deficit (gm/dl)†							
Primary cesarean section	42	1.2 ± 0.2	−1.4-3.8	58	1.4 ± 0.1	−0.9-4.1	0.23
Repeat cesarean section	56	1.1 ± 0.1	−1.5-3.8	44	1.0 ± 0.2	−1.2-4.8	0.42
TOTAL	98	1.2 ± 0.1	−1.5-3.8	102	1.2 ± 0.1	−1.2-4.8	0.59

*One subject excluded because of missing information.

†Difference between the hemoglobin at admission and the hemoglobin on the second postoperative day.

dom, $p = 0.92$). The duration of membrane rupture averaged 12.7 (SEM, 1.5) hours for the patients in the stapler group versus 10.4 (SEM, 1.0) hours for the patients with the standard technique ($t = 1.29$, 51 degrees of freedom, $p = 0.20$).

Blood loss and operating time according to the hysterotomy procedure. Subjective assessment by the obstetrician of total blood loss and of blood loss as a result of the hysterotomy led to lower estimates for the group with the Auto Suture Poly CS 57 disposable surgical stapler technique in primary as in repeat cesarean sections (Table II). However, the differences in mean blood loss between the two groups were rather small, varying between 52 and 109 ml according to the measure and the subgroup of subjects considered. Only the difference in the total blood loss between the two study groups reached statistical significance (mean ± SEM, 492 ± 24 vs 579 ± 38 ml, $p = 0.05$). The fall in the hemoglobin concentration from admission to the second postoperative day was very similar in the two groups and did not differ significantly from one group to the other. Only one patient, belonging to the standard group, required a perioperative transfusion.

The mean durations of the operative phases are shown in Table III. The time from uterine incision to closure of hysterotomy was not shortened by the use of the stapling device. Instead, all operative phases tended to be of longer duration with the use of the stapling device as compared with standard procedure. However, the largest difference observed reached only 5 minutes and concerned the total operating time in primary cesarean sections (42 vs 37 minutes, $p = 0.03$).

Exclusion from the stapled group of the 10 subjects for whom the staples were not used did not alter the results presented here.

Technical difficulties related to the use of the stapling device for hysterotomy. In 40 of the 88 patients (45.5%) for whom the Auto Suture Poly CS 57 disposable surgical stapler was used, the surgeons did not use all the staples available. The number of unused staples varied between two and 16 and averaged five. The reasons given for not using all staples were: no hold in the tissues (two subjects), stapler too long (35 subjects), staples not staying closed (two subjects), uterine tear while passing the stapler (one subject).

Accidental extension of the hysterotomy was reported in 37 (42%) patients. Significant bleeding originating from the hysterotomy extensions was observed in 62.2% of these 37 patients. The length of the extension ranged from 1 to 5 cm with an average of 2.9 cm. An extension of the hysterotomy occurred in 31% of the patients for whom all staples were used, whereas it was reported in 55% of those for whom some staples were not used. Accidental extension of the hysterotomy was not less frequent in the second half of the study (45.0%) than in the first (39.6%).

In six of 88 patients, bleeding from the hysterotomy occurred because a uterine vein was accidentally punctured by the retaining pin of the stapler.

No major complication or fetal injury was encountered with the use of the stapling device.

Postoperative febrile morbidity and hospital stay. Four (4.1%) patients from the stapler group and six (5.9%) from the standard hysterotomy group had fe-

Table III. Comparison of operating time between the two study groups

Operating time (min) and cesarean type	*Hysterotomy with stapling device*			*Standard hysterotomy*			*p Value*
	*No.**	*Mean ± SEM*	*Range*	*No.**	*Mean ± SEM*	*Range*	
From abdominal incision to birth							
Primary cesarean section	41	11.4 ± 0.5	5-18	57	10.1 ± 0.7	4-30	0.12
Repeat cesarean section	56	12.4 ± 0.6	6-33	43	11.7 ± 0.7	4-25	0.43
TOTAL	97	12.0 ± 0.4	5-33	100	10.8 ± 0.5	4-30	0.06
From uterine incision to closure of hysterotomy							
Primary cesarean section	41	13.1 ± 0.7	3-30	57	12.9 ± 0.8	5-35	0.84
Repeat cesarean section	56	12.6 ± 0.5	5-23	43	11.3 ± 0.6	4-21	0.09
TOTAL	97	12.8 ± 0.4	3-30	100	12.2 ± 0.5	4-35	0.35
From abdominal incision to closure of the wound							
Primary cesarean section	41	42.2 ± 1.8	20-73	57	37.0 ± 1.6	5-60	0.03
Repeat cesarean section	56	42.7 ± 1.7	20-79	43	42.0 ± 1.8	26-80	0.77
TOTAL	97	42.5 ± 1.2	20-79	100	39.2 ± 1.2	5-80	0.05

*One subject from the group with stapling device and two subjects from the group with standard hysterotomy are excluded because of missing information on operating times.

brile morbidity according to Monif's criteria (Fisher exact test, $p = 0.75$). In the stapler group, two of these cases of febrile morbidity were attributed to endometritis and two fevers were of unknown origin. In the group with the standard procedure, the fever was attributed to wound infection in two patients, to endometritis in one patient, and was of unknown origin in three subjects. The mean postoperative stay was identical in the two groups (mean ± SEM, 6.3 ± 0.1 days).

Comment

To our knowledge, this is the first randomized trial comparing the Auto Suture Poly CS 57 disposable surgical stapler with the standard procedure in low transverse hysterotomies for cesarean sections. Our results indicate that the operating times, the risk of febrile morbidity, and the postoperative hospital stay are not reduced with the use of the stapling device for this operation. Subjective assessments of blood loss were somewhat lower for patients in the stapler group. However, the differences were small and of questionable clinical significance. Moreover, when objectively estimated by the hemoglobin deficit measured on the second postoperative day, blood loss did not differ according to the hysterotomy technique.

Our results are only partly consistent with those published by Burkett et al.[4] In their retrospective study of 62 cesarean sections with staples and an equal number of cesarean sections without staples, these authors observed, as we did, a longer operating time in the primary cesarean sections with staples than in the nonstapled group. On the other hand, the deficit in hemoglobin reached statistical significance in their study, whereas it did not in ours. But the main difference in the results from the two studies concerns the effect of the stapling device on the risk of endometritis. Burkett et al.[4] reported a significant reduction in the frequency of endometritis from 22.5% in the nonstapled group to 6.4% in the stapled group. They could not provide an explanation neither for this large difference between the two study groups nor for the unusually high frequency of endometritis in their study population. The reduction of endometritis observed with the staples shortened the length of hospital stay and substantially reduced the costs related to the hospitalization in that group. Such benefits were not observed in our study population, in whom the overall endometritis rate was much lower (1.5%).

According to the obstetricians who used the stapler within our study, a traction applied on the lower uterine segment with an Allis forceps while introducing the stapler facilitated the procedure and allowed a more horizontal and thus a longer incision. Furthermore, there is probably room for improvement in the design of the stapling device. In our opinion, the most important complication related to the stapler is the bleeding that sometimes occurs at the site of the retaining pin. This pin is situated at the distal extremity of the stapling device, outside the last staple. Although the retaining pin is important to assure an adequate closure of the staples, its actual location outside the last distal staple is disturbing. If the lower uterine segment happens to be highly vascularized, this pin can puncture a uterine vein and cause substantial bleeding. This explains why the operator in 35 of our study patients considered the stapler "too long" and did not use all the staples available. In those cases, rather than using the whole length of the stapler and risking a uterine vessel puncture by the retaining pin, the operator chose to insert only part of the stapler in the uterus and to clip off the excess staples present at the medial extremity of each hemihysterotomy.

In conclusion, given the cost of the stapling device

and the absence of marked benefits from its use, our study does not support the routine use of the actual Auto Suture Poly CS 57 disposable surgical stapler in low transverse hysterotomies for cesarean sections.

REFERENCES

1. Adzick NS, Harrison MR, Flake AW, Glick PL, Bottles K. Automatic uterine stapling devices in fetal operation: experience in an primate model. Surg Forum 1985;36:479-81.
2. Adzick NS, Harrison MR, Glick PL, et al. Fetal surgery in the primate. III. Maternal outcome after surgical surgery. J Pediatr Surg 1986;21:477-80.
3. Bond SJ, Harrison MR, Slotnick RN, Anderson J, Flake AW, Adzick NS. Cesarean delivery and hysterotomy using an absorbable stapling device. Obstet Gynecol 1989;74: 25-8.
4. Burkett G, Jensen LP, Lai A, et al. Evaluation of surgical staples in cesarean section. AM J OBSTET GYNECOL 1989; 161:540-7.
5. Gibbs RS. Postpartum endometritis. In: Monif GRB, ed. Infectious diseases in obstetrics and gynecology. Philadelphia: Harper and Row, 1982:378.

The associated anomalies that determine prognosis in congenital omphaloceles

Marisa Tucci, MD, and Harry Bard, MD

Montreal, Quebec, Canada

Congenital abdominal wall defects such as omphaloceles can be recognized by fetal ultrasonography. To determine whether associated anatomic features may be useful in determining fetal prognosis, a retrospective study was performed over a 5-year period. There were 28 cases of omphalocele; 16 were larger than 5 cm and classified as giant, and the remainder were considered small. Eleven of the 12 infants with small omphaloceles survived with minimal neonatal complications. Ten of the 16 infants with giant omphaloceles died because of associated congenital anomalies. These were congenital heart disease, central nervous system malformations, and diaphragmatic hernias. This review suggests that the prognosis is good when a prenatal diagnosis of giant omphalocele is made and careful fetal ultrasonography, including echocardiography, does not identify heart, central nervous system, or diaphragmatic malformations, even when there is liver herniation into the omphalocele. (AM J OBSTET GYNECOL 1990;163:1646-9.)

Key words: Congenital abdominal wall defects, omphalocele, prenatal diagnosis

The incidence of omphaloceles is reported to be 1 in 4000 to 5000 live births.[1] The current widespread use of prenatal fetal ultrasonography enables the early detection of abdominal wall defects, the most common of which are omphaloceles. It also permits the possible identification of other fetal anomalies frequently associated with this defect.[2] However, for the antenatal findings to be better used in predicting prognosis, it is important to have information on the short-term outcome of these infants, especially those with large omphaloceles. A high incidence of major congenital anomalies has been reported in infants with giant omphaloceles; the prognosis has been considered especially poor when there is liver herniation into the omphalocele.[3-5]

The object of this study was to compare the infants with congenital omphaloceles who survived with those who died. On the basis of the differences found, this could lead to a better identification during prenatal ultrasonography of those fetuses with a good prognosis. This information could be particularly important to obstetricians, pediatric surgeons, and neonatologists.

From the Perinatal Service, Ste-Justine Hospital, Department of Pediatrics, University of Montreal.
Received December 13, 1989; revised July 5, 1990; accepted July 16, 1990.
Reprint requests: Marisa Tucci, MD, Hôpital Ste-Justine, 3175 Chemin Côte Ste-Catherine, Montreal, Quebec, Canada, H3T 1C5.
6/1/23779

Methods

A total of 28 infants born with omphaloceles over a 5-year period (1984 to 1988, inclusively) were identified at our institution. Sainte-Justine Hospital is a regional university perinatal center. This tertiary care center specializes in high-risk obstetrics and has approximately

Table I. Infants with giant omphalocele who did not survive ($n = 10$)

Gestational age classification[6]	*Presence of liver*	*Presence of diaphragmatic hernia*	*Other important major malformations*	*Age at death (days)*	*Karyotype*
TAGA	+	+	–	25	NA
TSGA	+	–	Tetralogy of Fallot Cerebral frontal hypoplasia*	27	N
PoAGA	–	–	Cerebral atrophy† Cyst third ventricle† Hypoplasia corpus callosum† Large ASD	13	N
PrAGA	+	+	Complex cardiopathy (Tetralogy of Fallot, PDA, large VSD, bicuspid pulmonary valve)‡	1	N
PrAGA	+	–	Diffuse cerebral hypoplasia† PDA	1	N
TAGA	–	–	Olfactory tract and bulb agenesis‡ Bilateral hippocampal hypoplasia Corpus callosum hypoplasia Large ASD and VSD Double-outlet right ventricle Anomalous tricuspid valve Sacral agenesis Complete bilateral cleft palate	1	NA
TAGA	+	–	Type III esophageal atresia‡ VSD, interventricular septal aneurysm, biventricular hypertrophy	370	N
PrAGA	+	–	Left lung hypoplasia‡ Leukomalacia of cerebral hemispheres	15	N
TAGA	–	–	Total anomalous venous return, multiple VSD, dextrocardia	93	NA
TSGA	+	+	–	1	NA

N, Normal; *NA*, not available; *PDA*, patent ductus arteriosus; *ASD*, atrial septal defect; *VSD*, ventricular septal defect; *TAGA*, term appropriate for gestational age; *TSGA*, term small for gestational age; *PoAGA*, postterm appropriate for gestational age; *PrAGA*, pre-term appropriate for gestational age.

*Anomaly noted on cerebral ultrasonography (no autopsy performed).

†Anomalies noted on cerebral computerized tomography (no autopsy performed).

‡Anomalies noted on autopsy.

4000 deliveries per year, of which one third are high-risk deliveries. It is a center to which mothers and newborns are transferred from a variety of institutions ranging from small rural hospitals to other teaching centers in the province. The hospital is the reference center for about 40,000 deliveries per year, including a significant number of congenital anomalies necessitating surgical intervention. In the majority of pregnancies in the province of Quebec (90%) at least one ultrasonographic examination is performed routinely,

Table II. Infants with giant omphalocele who survived ($n = 6$)

Gestational age classification[6]	*Presence of liver*	*Presence of diaphragmatic hernia*	*Other important major malformations*	*Karyotype*
TAGA	+	–	–	N
TAGA	+	–	–	N
TAGA	–	–	–	NA
TAGA	–	–	–	Trisomy 21
TSGA	+	–	–	N
TAGA	+	–	–	N

N, Normal; *NA*, not available; *TAGA*, term appropriate for gestational age; *TSGA*, term small for gestational age.

usually between 16 and 20 weeks' gestation with other ultrasonographic examinations if indicated.

A retrospective analysis of the charts of 28 cases of omphalocele was performed. The elements most specifically sought were size of the omphalocele and its characteristics, associated malformations, and outcome. Cardiac anomalies were diagnosed by echocardiography. The infants were classified in accordance with the University of Colorado intrauterine growth charts.[6] These infants were divided into two groups: those with giant and those with small omphaloceles. Omphaloceles were classified as giant if ≥5 cm in diameter.[4, 5]

Results

A totals of 28 cases of central omphaloceles were reviewed. Among these, 12 were <5 cm and could therefore be labeled as small omphaloceles, whereas 16 were deemed giant because they were ≥5 cm in diameter. There was no significant predominance of either sex.

In the group of 12 infants with small omphaloceles, eight infants were born at term (with one small for gestational age and seven appropriate for gestational age), one was postterm appropriate for gestational age, and three were preterm appropriate for gestational age. Among the three preterm infants, the most premature was born at 29 weeks' gestation; the other two were born at 34 and 35 weeks' gestation. Meckel's diverticulum was noted in four of these infants. Ileal atresia was present in one case. Beckwith-Weidemann syndrome with macroglossia and hypoglycemia was suspected in one infant. One infant had trisomy 21. Only one infant died in this group of 12 with small omphaloceles. This unpreventable death was not related to the presence of an omphalocele. The infant's mother had premature rupture of the membranes at 19 weeks' gestation. The infant was born at 29 weeks' gestation and had evidence of lung hypoplasia. Unfortunately, autopsy was refused by the parents. The other 11 infants in this group with small omphaloceles had no significant problems and had excellent outcomes with no complications.

There were 16 infants in our study who were classified as having giant omphaloceles. In this group 12 were term (nine were appropriate for gestational age and three were small for gestational age), one was postterm appropriate for gestational age, and three were preterm appropriate for gestational age. Among the three preterm infants, the most premature was born at 29 weeks of gestation; the other two were born at 34 and 35 weeks of gestation. Ten of these 16 infants with giant omphaloceles died. Table I lists the major anomalies in these 10 infants. Five had severe congenital heart defects. There were three infants with diaphragmatic hernias and two with central nervous system malformations. One infant died of respiratory complications after abdominal wall closure and had no evidence of other associated malformations.

Among the six infants with giant omphaloceles who survived, none had associated severe malformations. Table II lists the major features found in those who survived. One infant in this group had trisomy 21. Four of the infants in the group of six survivors had evidence of liver herniation into the omphalocele.

Comment

The purpose of this study of omphaloceles was to redefine or confirm the influence of various features on the outcome of affected infants. The size of the defect is a factor known to be of prognostic importance. Previous studies have already ascertained that small omphaloceles are rarely associated with other severe anomalies and have an excellent prognosis. The prognosis of newborn infants with giant omphaloceles is not as good. The outcome in these infants is influenced by several factors, in particular the type of malformations frequently found to be associated with large omphaloceles.[4, 5, 7] All but one of the infants with small omphaloceles survived, whereas fewer than half of the infants with giant omphaloceles survived. Those with giant omphaloceles who died had at least one major congenital anomaly in addition to omphalocele. Those who survived had no other major anomalies.

It is evident that the presence of a giant omphalocele with multiple anomalies is indicative of a poor prognosis. Among the numerous anomalies found in these

infants, one that is particularly indicative of a grave prognosis is the presence of the diaphragmatic hernia. All three infants in our study who had giant omphaloceles and diaphragmatic hernias did not survive. The two organ systems that, when affected, seemed to play a more important role in determining outcome were the brain and heart. All infants with either central nervous system anomalies or major congenital heart defects died.

Studies in the past have suggested that liver herniation is associated with a neonatal death rate as high as 88%.[3,5] It would be logical to state that the presence of liver among the herniated viscera may indeed be an indication of the importance of the defect. In this study, the presence of a herniated liver alone was not of prognostic importance in relation to survival. In fact, on the basis of this small series, a fetus with a giant omphalocele without any other major anomaly, even when the liver is herniated, can have a good prognosis.

Whenever a congenital omphalocele is suspected, targeted prenatal ultrasonographic examination by qualified obstetricians or radiologists to recognize detailed fetal anomalies becomes crucial. With the use of fetal echocardiography, recognition of congenital heart defects is possible and could allow antenatal detection of severe cardiac malformations.[8] A methodical, rigorous use of imaging technology by qualified individuals would permit the identification of those aforementioned features that are indicative of a graver prognosis. Other diagnostic tests such as amniocentesis and fetal blood sampling by cordocentesis may be appropriate. Consequently, realistic expectations can be explained to parents, and a rational management for the further conduct of the pregnancy can be formulated.

In summary, the features identified as having the most influence on the outcome of an infant with an omphalocele were (1) whether the ompalocele was qualified as giant, (2) the existence of multiple associated congenital anomalies (especially central nervous system or congenital heart defects), and (3) the presence of a diaphragmatic hernia. Despite the size of the defect, even when the liver is herniated, the absence of other associated major malformations could be indicative of good prognosis.

REFERENCES

1. Baird PA, McDonald EL. An epidemiologic study of congenital malformations of the anterior abdominal wall in more than a million consecutive live births. Am J Hum Genet 1981;33:470-8.
2. Petres RE, Redwine FO. Ultrasound in the intra-uterine diagnosis and treatment of fetal anomalies. Clin Obstet Gynecol 1982;25:753-71.
3. Sermer M, Benzie RJ, Pitson L, Carr M, Skidmore H. Prenatal diagnosis and management of congenital defects of the anterior abdominal wall. Am J Obstet Gynecol 1987;156:308-12.
4. Mayer T, Black R, Matlak MJ, Johnson DG. Gastroschisis and omphalocele. Ann Surg 1980;192:783-7.
5. Hershenson MB, Brouillette RT, Klemka L, Raffensperger JD, Poznanski AK, Hunt CE. Respiratory insufficiency in newborns with anterior wall defects. J Pediatr Surg 1985;20:348-53.
6. Battaglia F, Lubchenco LO. A practical classification of newborn infants by weight and gestational age. J Pediatr 1967;71:159-63.
7. Hadziselimovic F, Duckett JW, Snyder III HM, Schnaufer L, Muff D. Omphalocele, cryptorchidism and brain malformations. J Pediatr Surg 1987;22:854-8.
8. Allan LD. Diagnosis of fetal cardiac abnormalities. Arch Dis Child 1989;64:964-8.

Ultrasonographic assessment of placental abnormalities

Eric Jauniaux, MD,* and Stuart Campbell, FRCOG
London, England

Current ultrasonographic techniques offer a novel approach for the identification of a wide variety of placental abnormalities usually described postnatally by the pathologist. Placental vascular lesions, placental tumors, and abnormal placentation are potentially associated with perinatal complications and their diagnosis in utero may influence the pregnancy management. An ultrasonographic classification of placental lesions that is based on their location, size, echogenicity, and number is proposed. Repeated ultrasonographic examination, together with biologic investigations, is important for the prenatal differential diagnosis of most of these lesions and for full understanding of their pathophysiologic characteristics and significance. (AM J OBSTET GYNECOL 1990;163:1650-8.)

Key words: Ultrasonography, placenta, prenatal diagnosis

Recognition of normal and abnormal placental features is a prerequisite for the interpretation of ultrasonographic images. However, despite >20 years of experience in examination of the placenta in utero by ultrasonography, controversies still exist regarding the terminology and the significance of different placental ultrasonographic features.

The purpose of this work is to review the different placental ultrasonographic abnormalities together with their pathophysiologic and clinical implications.

Placental development and maturation

The development of the placenta has always attracted considerable interest from both anatomists and ultrasonographers. Combined studies using transvaginal ultrasonography, hysteroscopy, chorionic villus sampling, and hysterectomy specimens from the first trimester of pregnancy have recently indicated the absence of continuous blood flow in the intervillous space before 12 weeks of gestation.[1-3] During the first trimester of pregnancy, the placenta is bathed by a clear fluid probably composed of maternal plasma and uterine gland secretions.[1-3]. After 12 weeks the trophoblastic plugs in the spiral arteries that are still remaining from the first extravillous trophoblastic wave no longer obliterate the uteroplacental arteries, and a real circulation is then established.[1]

Ultrasonographic evidence of placental textural changes in vivo were reported in early studies during the second and third trimesters.[4-6] A ultrasonographic classification system for grading placentas in utero was developed by Grannum et al.[7] The placental maturational changes were determined by use of a contact B-scanner and graded from 0 to III. These placental changes were correlated with fetal pulmonic maturity evaluated in utero by amniotic fluid lecithin/sphingomyelin (L/S) ratios obtained by amniocentesis. An ideal correlation of 100% was found between placental grade III and mature L/S ratios, suggesting that ultrasonographic placental grading might replace amniocentesis as a standard test to assess fetal pulmonic maturity. Other authors[8, 9] presented similar results using real-time ultrasonography. However, larger studies showed that the sensitivity of the placental grading system as an indicator of pulmonic maturity was poor.[10-14] In addition, this system falsely predicted fetal lung maturity in 8% to 42% of the cases and was therefore not accurate enough to replace amniocentesis for this purpose.[10-14] A modified classification that is based on multiple placental ultrasonographic sections was also proposed, suggesting that placentas should be considered mature only when grade III changes are present in all sections.[15]

Various interpretations of the placental structural changes, interobserver variation in scanning technique, and heterogeneity of the study groups are probably

From the Department of Obstetrics and Gynecology, King's College School of Medicine and Dentistry, University of London.
Reprint requests: Eric Jauniaux, MD, Department of Obstetrics and Gynecology, University Hospital Erasme, Free University of Brussels (ULB), 808 Route de Lennik, B-1070 Brussels, Belgium.
**Research Fellow from the Free University of Brussels (ULB), Brussels, Belgium, supported by the British Council (Sir Alexander Fleming Award 1989).*
6/1/22989

Table I. Differential diagnosis of principal placental lesions reported in the literature

Location	*Sonographic features*	*Pathologic classification*
Fetal plate	Multiple sonolucent areas limited to the placental periphery (Fig. 1, *A*)	Circumvallate placenta
		Circummarginate placenta
	Single sonolucent or hypoechoic area surrounded by a thin membrane (Fig. 1, *C* and *F*)	Subamniotic cyst
		Old subamniotic hematoma
	Single hyperechoic area surrounded by a thin membrane (Fig. 1, *E*)	Recent subamniotic hematoma
	Well-circumscribed heterogeneous mass protruding into the amniotic cavity (Fig. 2)	Chorioangioma
Placental tissue	Small sonolucent area in the center of the cotyledon	Centrocotyledonary cavity
	Large sonolucent area (Fig. 3, *A*)	Large avillous zone (cavern)
		Septal cyst
		Early stage of thrombosis formation
	Large hyperechoic area (Fig. 3, *C* and *E*)	Old thrombosis
		Infarct
	Multiple sonolucent areas of various sizes and shapes (Fig. 4)	Hydatidiform transformations
Maternal plate	Large hyperechoic area	Recent retroplacental hematoma
	Large sonolucent or hypoechoic area	Old retroplacental hematoma

responsible for the controversies generated by the placental grading system. Nevertheless, all these studies have highlighted that complicated pregnancies have a different maturation pattern than normal pregnancies. This important clinical finding was confirmed by further selective studies. Pregnancies complicated by chronic hypertension or preeclampsia and/or intrauterine growth retardation present an accelerated placental maturation, whereas diabetes and maternal-fetal rhesus incompatibilities are associated with delayed placental maturation.[16] Earlier maturational changes were also described in twin gestations, as compared with singleton gestations,[17] and after exposure to maternal smoking.[18, 19] Pregnant women with mature placental appearance (grade III) on ultrasonography between 34 and 36 weeks' gestation have an increased risk of problems during labor, and their babies have an increased risk of low birth weight, intrapartum distress, and perinatal death.[18] This study also demonstrated that there was a significant decrease in the risk of perinatal death when the grading was known by the clinician responsible for care.[18]

Most of the above-mentioned studies refer to placental senescence or aging to explain the placental maturational changes observed by ultrasonography. Light or electron microscopic study of villi from term placenta does not indicate an aging process.[20] Furthermore, the total placental deoxyribonucleic acid levels continue to rise in a linear fashion until, and beyond, the fortieth week of gestation.[21] Therefore the placental ultrasonographic changes should not be considered as the morphologic hallmarks of placental senescence.

Several authors have attempted to reproduce in vitro the ultrasonographic morphologic features observed in vivo.[4, 5, 22] In early studies the placentas were not always fixed immediately and some changes observed in vivo were not found in vitro; furthermore, new features were probably created during placental collapse.[4, 5] More satisfactory results were obtained with the use of a perfusion system in vitro, to avoid placental collapse.[22] Sonolucent areas or central echo-free spaces have been correlated with avillous zones, and the peripheral echoes with septal calcification and fibrin deposition.[4, 5]

Classification of placental ultrasonographic lesions

Many inaccurate and misleading expressions have been used by sonographers to described placental pathologic lesions. This is probably because of the fact that little attempt has been made to compare ultrasonographic and postpartum findings. The location, the shape, and the echogenicity of the lesion and the number of lesions are the principal ultrasonographic features for the prenatal differential diagnosis of placental abnormalities (Table I).

Placenta extrachorialis. Placenta extrachorialis is characterized by a transition of membranous to villous chorion within the placental disk at a variable distance from the placental edge.[20, 23, 24] Between 18% and 30% of placentas are of the extrachorial variety.[20] Two anatomic entities have been described: circummarginate placentas, which have a flat ring of membranes made up only of amnion and chorion, and circumvallate placentas, which have a raised, often rolled ring of membranes, containing amnion, chorion, and decidual tissue.

Circummarginate placentas are practically asymptomatic.[20] In contrast, circumvallate placentas are associated with a relatively high rate of premature rupture of the membranes, antepartum bleeding, and preterm onset of labor.[20, 23] These complications are

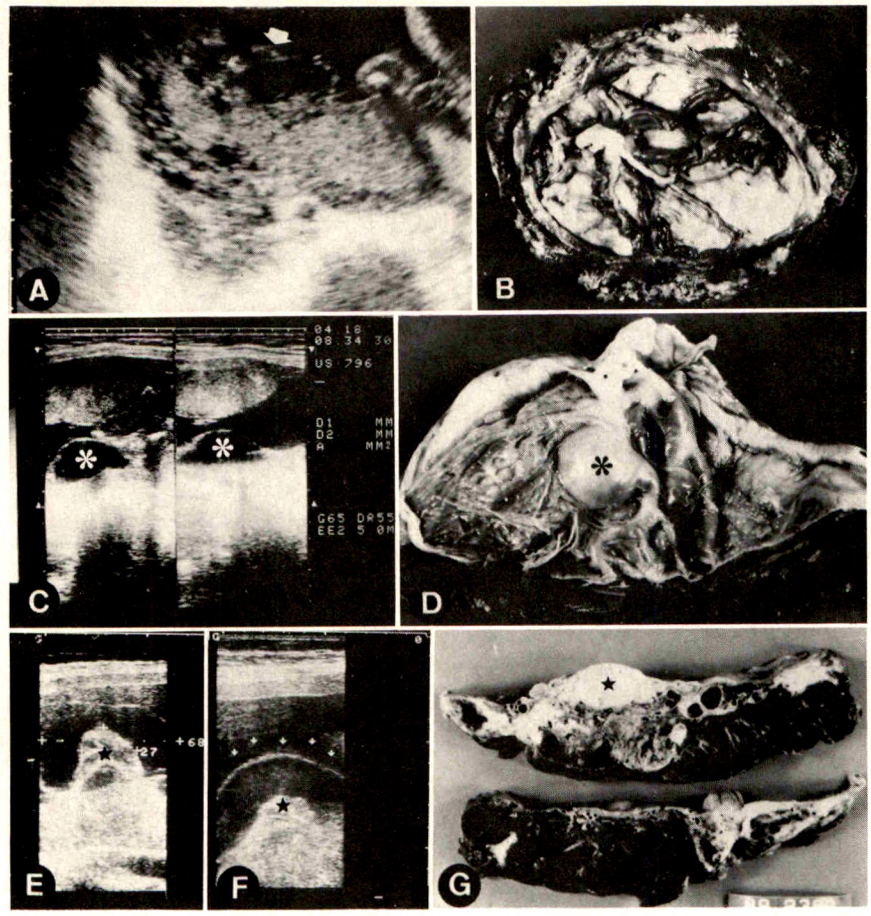


Fig. 1. A, Transverse scan at 20 weeks of gestation of the marginal zone of the placenta, showing sonolucent areas over the fetal plate *(arrow)*. These abnormalities were found only near the placental edge. **B**, Pathologic examination demonstrated a circumvallate placenta. **C**, Transverse and longitudinal scans of a single sonolucent subamniotic area *(asterisk)* at 32 weeks corresponding to a subamniotic cyst (**D**) *(asterisk)*. **E** and **F**, Longitudinal sonograms at 24 and 32 weeks, respectively, showing hyperechogenic placental lesions on the top of the fetal plate *(stars)*, surrounded by a thin membrane *(arrows)*. The lesion becomes less echogenic as the clot resolves. **G,** Pathologic examination revealed an old subamniotic hematoma *(star)*.

probably due to a lack of adaptation of the rigid placental edge with tearing of membranes as the uterine wall stretches in the second half of gestation.

Multiple subamniotic sonolucent areas of various sizes and shapes, located in the periphery of the placenta (Fig. 1, *A* and *B*) are the main ultrasonographic features of this form of placentation.[25]

Cytotrophoblastic cysts. Cytotrophoblastic cysts usually have a single, round or oval cavity, containing a gelatinous material.[20, 23, 24] They can be divided into septal cysts when they are located within the placental tissue and subchorionic cysts when they are on the fetal surface beneath the chorionic vessels.[23, 24]

These cysts are isolated from the placental circulation and appears ultrasonographically as single sonolucent areas (Fig. 1, *C* and *D*) showing no blood flow on real-time imaging.[26] Cytotrophoblastic cysts are found in 20% of placentas at term.[20] Their incidence in increased in pregnancies complicated by diabetes mellitus or maternal-fetal rhesus incompatibility.[20]

Subamniotic hematomas. These lesions are secondary to the rupture of a fetal vessel before or during delivery and are located beneath the amniotic layer covering the fetal plate.[20] Their incidence is unknown but most of them are found in third-trimester placentas and are thought to occur during delivery as a result of excessive traction on the umbilical cord.[20] Subamniotic hematomas that develop during the second or third trimester are sometimes complicated by fetal growth retardation and abnormal Doppler measurements.[27, 28]

Ultrasonographically, they appear as a single mass protruding from the fetal plate and surrounded by a thin membrane. The newly formed clot is echogenic

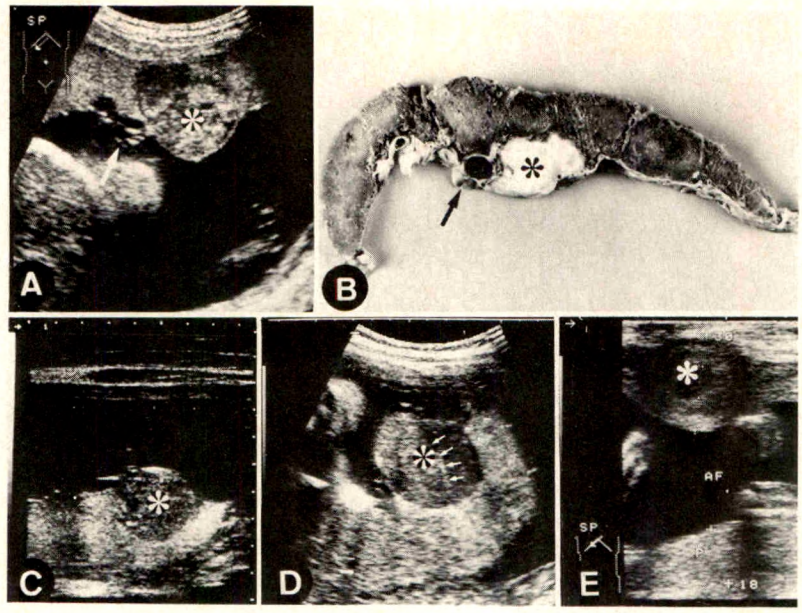


Fig. 2. A, Heterogeneous placental mass at 32 weeks *(asterisk)*, protruding from the fetal plate near the cord insertion *(arrow)*. The pregnancy was complicated by polyhydramnios. **B**, Pathologic examination demonstrated a chorioangioma *(asterisk)* showing generalized degenerative changes. **C**, Longitudinal ultrasonogram of a hypoechoic placental mass *(asterisk)* at 18 weeks gestation. **D**, At 30 weeks half of the lesion *(asterisk)* was more echogenic. At delivery the lesion corresponded to a large chorioangioma partly grooved by bands of fibrous tissue. **E**, Ultrasonographic appearance of a uterine leiomyoma *(asterisk)* at 20 weeks of gestation, for comparison.

(Fig. 1, *E*), but the lesion becomes less so as the clot resolves (Fig. 1, *F* and *G*).

Chorioangiomas. Chorioangiomas occur in 0.5% to 1% of placentas examined at term.[22, 23] Most chorioangiomas are encapsulated, single, small, round, and intraplacental.[20, 23] Large tumors can be of variable shape, divided by fibrous septa, and they often protrude from the fetal surface near the cord insertion.[20, 23] Degenerative changes such as necrosis, calcification, hyalinization, or myxoid changes are frequently present in large tumors. Chorioangiomas are associated with an increased incidence of polyhydramnios and fetal growth retardation.[20, 27] Large tumors (>5 cm in diameter) can also be complicated by fetal cardiac failure with hydrops because of the shunting of blood through the tumor.[20]

On ultrasonographic, only large chorioangiomas protruding into the amniotic cavity have been documented. They are well circumscribed, have a different echogenicity from the rest of the placental tissue (Fig. 2, *A* and *B*), and are easily detectable early in pregnancy by ultrasonographic.[27, 29-31] The echogenicity varies according to the degree of degenerative changes present in the tumor (Fig. 2, *C* and *D*).

Caverns. These lesions may vary from small sonolucent areas to large sonolucent spaces (Fig. 3, *A*). Small echo-free spaces in the center of the cotyledon have been described in normal third-trimester mature placentas (grade III of Grannum's classification). These small cavities within the cotyledons are secondary to the dispersion of the free-floating terminal villi by maternal arterial jets of blood entering the intervillous space.[22]

Larger intraplacental sonolucent spaces are found in 67% of the placentas examined by ultrasonography from the first half of pregnancy until delivery.[32] They contain turbulent blood flow on real-time imaging and their shape can be modified by maternal position or by uterine contractions.[26] Histologically, these lesions correspond to large avillous areas surrounded by normal villi. They are probably a nonpathologic variation of the normal placental anatomic structure that develops from early in pregnancy.[26] They have been described by pathologists as hemorrhagic areas[20] or as caverns,[23] sometimes containing semifluid or fluid blood.

Thrombosis. Placental thrombosis is the result of focal coagulation of blood in the intervillous spaces and usually occurs during the third trimester.[20, 23, 24] Intervillous thromboses are found in about 40% of placentas and contain an admixture of fetal and maternal blood.[20, 24] The incidence of the lesion is increased in pregnancies complicated by rhesus isoimmunization.[20]

Large hypoechoic areas with low flow laterally and relatively high flow in the central part on real-time imaging can be observed in the early stages (Fig. 3, *A*) of the development of an intervillous thrombosis.[26, 32, 33] Abnormal hemodynamic flow in the intervillous space

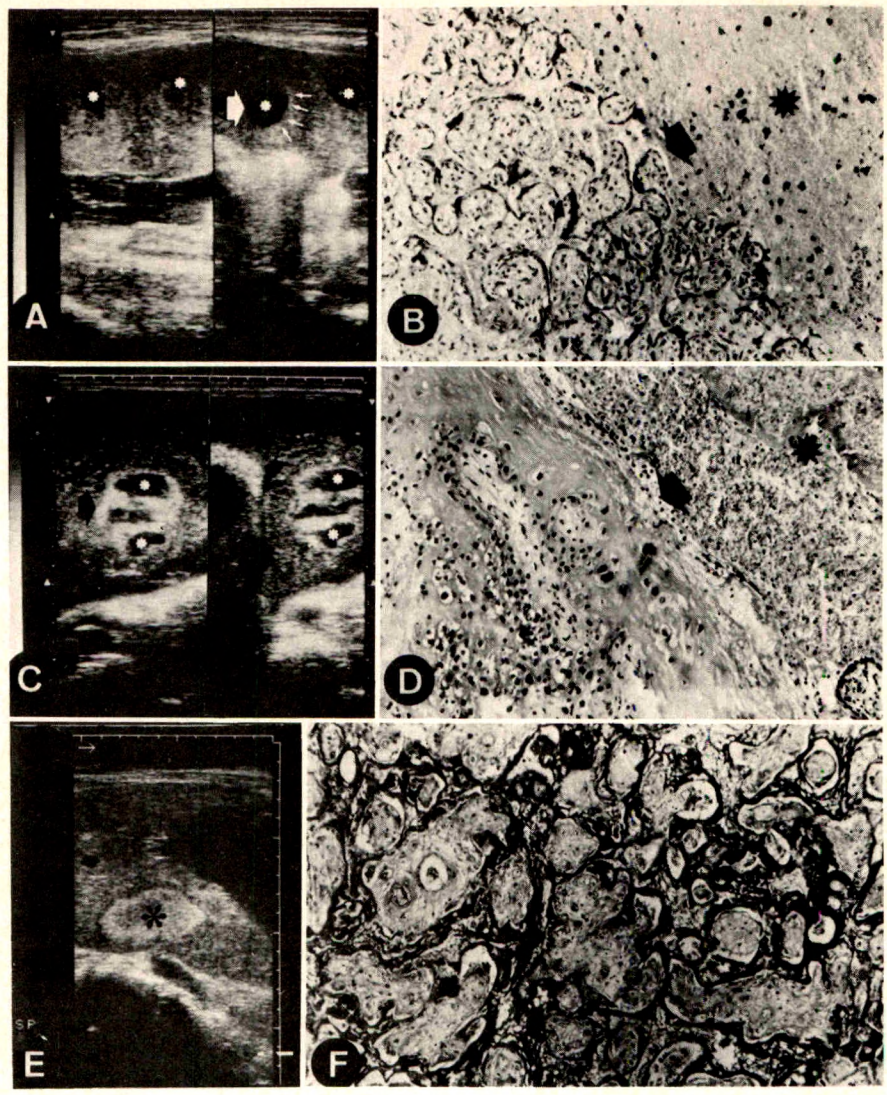


Fig. 3. A, Ultrasonograms at 35 weeks showing small and large placental sonolucent spaces *(asterisks)*, containing turbulent blood flow. Note the increase echogenicity of the surrounding villi *(small arrows)*. **B**, Histologic section at the level of the *large arrow* showing a recent intervillous thrombosis *(asterisk)* and normal villi *(arrow)*. The villi surrounding the lesion at the level of the *small arrows* were compressed and infarcted. (Hematoxylin and eosin. ×50.) **C**, Ultrasonograms at 39 weeks showing a large placental lesion *(asterisk)* corresponding (**D**) to an organized intervillous thrombosis *(asterisk)* with extensive fibrin deposition *(large arrow)* in periphery. (Hematoxylin and eosin. ×50.) **E**, Large placental hyperechoic area *(asterisk)* located near the basal plate at 32 weeks of gestation corresponding (**F**) to a chronic infarct. (Hematoxylin and eosin. ×50.)

may result from the failure of the cotyledon to expand in response to the increasing flow of the corresponding uteroplacental artery and compression of the surrounding villi with gradual atrophy as fibrin accumulates in the periphery. This process causes a progressive increase in the echogenicity of the lesion (Fig. 3, *C* and *D*), thus distinguishing it from a placental cavern. Finally, the maternal blood coagulates in the placental tissue, focally obliterating the intervillous circulation. This failure to accommodate may be secondary to inadequate venous drainage from the cotyledon or to villous damage related to maternal-fetal incompatibility.[20, 23]

Intervillous thrombosis must be distinguished from subchorial thrombosis as a result of pooling and stasis of maternal blood beneath the chorionic plate or in the marginal area of the intervillous chamber.[26, 34] Ultrasonographically, these lesions appear as sonolucent space of variable size containing turbulent blood flow on real-time imaging.[26] Their appearance may change from one examination to another as progressive fibrin deposition leads to classic subchorial or marginal thrombosis described by the pathologists.[20, 23]

Infarcts. Placental infarcts are the result of obstruction of a uteroplacental artery leading to focal degeneration of the overlying villous tissue.[20, 23] They are

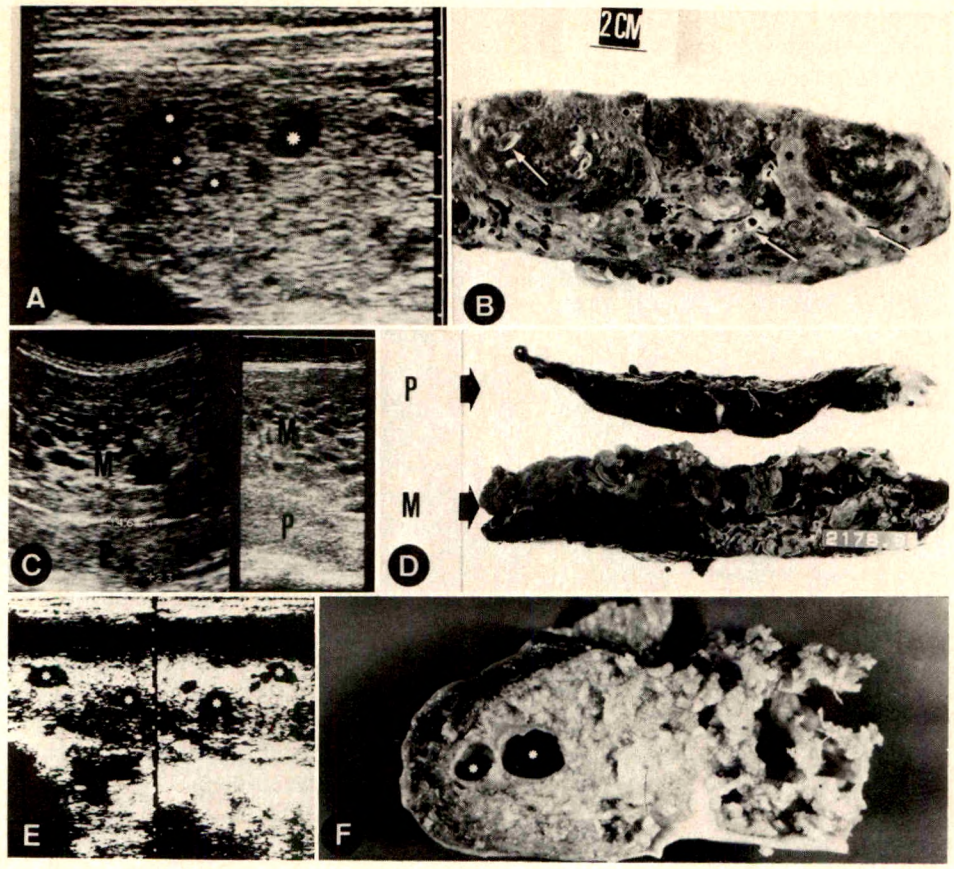


Fig. 4. A, Longitudinal ultrasonogram at 22 weeks of gestation showing an enlarged placenta containing multiple sonolucent spaces *(asterisk)*. The fetus was anatomically normal. **B**, Pathologic examination demonstrated diffuse mesenchymal hyperplasia with myxoid degeneration of the villous stem and dilatation of the main fetal vessels *(arrows)*. **C**, Ultrasonogram at 25 weeks of a molar mass *(M)* and a normal placenta *(P)* in a twin pregnancy combining a classic mole and a normal placenta and fetus. **D**, Pathologic examination confirmed the presence of a normal placenta *(P)* and a complete mole *(M)*. **E**, Transverse ultrasonograms of an enlarged placenta containing multiple sonolucent spaces of various sizes and shapes *(asterisk)*. The pregnancy was complicated by severe asymmetric fetal growth retardation, oligohydramnios, and early pregnancy-induced hypertension. **F**, Pathologic and cytogenetic investigations revealed a triploid syndrome with focal swelling of the villous tissue *(asterisk)*.

usually located near the maternal plate.[20, 23] Small infarcts are found in about 25% of placentas from uncomplicated pregnancies.[20] The incidence of placental infarction is significantly increased in pregnancies complicated by preeclampsia or essential hypertension[20, 23, 24] and is directly related to the severity of the disease.[20, 23] In these maternal hypertensive disorders infarction is extensive and involves more than 10% of the placental tissue.[20] Large placental infarcts are associated with an increase in perinatal mortality and intrauterine growth retardation.[20, 23, 24]

Ultrasonographically, placental infarcts appear as large intraplacental areas that are irregular and hyperechoic in the acute stage[26] and isoechoic in more advanced stages (Fig. 3, *E* and *F*).

Hydatidiform transformation. Molar or hydatidiform transformation of the villous tissue (Fig. 4, *A*, *C*, and *E*) is a common finding in placental trophoblastic tumors. The principal histologic feature of this group of tumors is trophoblastic hyperplasia. Trophoblastic tumors are classified on a cytogenetic and morphologic basis into complete and partial mole.[35, 36]

Complete or classic hydatidiform moles are characterized by generalized swelling of the villous tissue, trophoblastic hyperplasia, and no embryonic or fetal tissue.[35, 36] Complete moles are almost always diploid with a 46,XX chromosomal constitution in 90% of cases and a 46,XY karyotype in 10% of cases.[37] About 15% to 20% of complete moles can become invasive and metastasize.[38] Ultrasonographically, the uterus is filled with sonolucent spaces ("snowstorm" appearance) of various sizes and shapes without embryonic or fetal material.[34] However, molar transformation of one ovum in a dizygotic twin pregnancy has been reported.[39, 40] In these cases ultrasonographic or pathologic examination shows a molar placental mass together with normal placenta and fetus (Figs. 4, *C* and *D*).

Partial hydatidiform moles are characterized by focal

Table II. Review of perinatal features of 35 cases of triploid syndromes diagnosed in second and third trimesters of pregnancy (references 43 to 54)

A. Maternal data (No. = 20)	
Mean maternal age (yr)	25.95 ± 4.91
Mean gravidity	2.1 ± 0.89
Mean parity	0.75 ± 0.83
B. Mean gestational age at diagnosis (wk) (No. = 33)	21.12 ± 4.31
C. Maternal complications (13 cases)	28.6%
6 Pregnancy-induced hypertension	
4 Bilateral multicystic ovaries	
3 Hyperemesis gravidarum	
3 Vaginal bleeding	
D. Ultrasonographic findings	
Placenta (No. = 34)	
Normal in 15 cases	44.1%
Abnormal in 19 cases	55.9%
4 Hydropic (enlarged)	
10 Molar transformations	
5 Hydropic and molar transformations	
Fetus (No. = 33)	
Normal in 3 cases	9.1%
Abnormal in 30 cases	90.9%
18 IUGR	
4 Malformations	
8 IUGR and malformations	
Amniotic fluid (No. = 28)	
Normal in 15 cases	53.6%
Abnormal in 13 cases	46.4%
9 Oligohydramnios	
4 Polyhydramnios	
E. Laboratory findings	
Maternal serum hCG (No. = 10)	
Elevated in 9 cases and normal in 1 case	
Maternal serum AFP (No. = 14)	
Elevated in 13 cases and normal in 1 case	
Amniotic fluid AFP (No. = 15)	
Elevated in 6 cases and normal in 9 cases	
F. Pathologic findings	
Placenta (No. = 31)	
Normal in 12 cases	38.7%
Abnormal in 19 cases	61.3%
9 Molar transformations	
12 Trophoblast hyperplasia and/or scalloped outline	
Fetus (No. = 26)	
Normal in 2 cases	7.7%
Abnormal in 24 cases	92.3%
21 Small for dates	
13 External defects	
10 Internal abnormalities	
G. Cytogenetic findings (No. = 35)	
69,XXX (20 cases)	
69,XXY (13 cases)	
69,XYY (no cases)	
70,XXYY (1 case)	
46,XY/69,XXY (1 case)	

IUGR, Intrauterine growth retardation; *No.*, number of cases among the 35 cases reviewed with the corresponding information; *hCG*, human chorionic gonadotropin; *AFP*, α-fetoprotein.

swelling of the villous tissue, focal trophoblastic hyperplasia, and embryonic or fetal tissue.[35, 36] The most common karyotype found in these cases is triploidy. This is a common chromosomal anomaly accounting for 1% to 2% of all clinically recognized human conceptions.[41, 42] There is a strong correlation between the origin of the extra set of chromosomes, the degree of the placental molar change, and the survival rate of the pregnancies.[42] All cases with an extra paternal set (73%) of chromosomes have significant molar changes (Fig. 4, *E* and *F*) while only 14% of cases with an extra maternal set (27%) have significant molar changes.[42] Placentas with nonmolar changes are associated with a better fetal survival and are more likely to be found near term.[42]

Table II summarizes the perinatal data of 35 cases of triploidy reported in the literature.[43-54] Only triploidy syndromes confirmed by chromosomal analysis were included. This review indicates that 55.8% of the placentas in triploidy have hydropic and/or molar changes on ultrasonography. The fetus presents severe growth retardation and/or major congenital defects in 90.9% of the cases and the amniotic fluid volume is abnormal in 46.4%. The ultrasonographic features correlated well with the morphologic findings reported in these cases and in more selective pathologic studies.[42] This review is not exhaustive and most of these studies are incomplete; in particular, microscopic findings and laboratory investigations were not constantly reported. Nevertheless, published biologic findings indicate that most of the cases of triploidy are first seen with elevated levels of maternal serum human chorionic gonadotropin and α-fetoprotein even when they are not associated with major congenital fetal defects.

Cases of diploid partial moles were recently described. They are characterized by diffuse molar placental changes associated with a normal fetus and elevated levels of human chorionic gonadotropin and α-fetoprotein.[55] Pathologically, areas bearing characteristic molar transformation (diploid cytogenetic constitution) interdigitate with unaffected placental areas.[55, 56]

Molar changes of the placenta are not always pathognomonic of trophoblastic disorders and can be found in other placental pathologic conditions such as benign diffuse mesenchymal hyperplasia (Fig. 4, *A* and *B*) or in prolonged placental retention in utero after fetal death.[26, 57]

Retroplacental hematomas. The diagnosis of abruptio placentae during the second half of pregnancy is based on the clinical triad of pain, uterine rigidity, and vaginal bleeding, and ultrasonographic investigations can be used only in nonacute cases to confirm clinical diagnosis and to exclude presence of placenta previa.[58]

Retroplacental hematomas cause a wide spectrum of ultrasonographic features depending on the location of the lesion and on the degree of organization of the blood clot. Acute hemorrhage is hyperechoic to isoechoic, as compared with placental tissue, while resolving hematomas are hypoechoic.[59]

Other placental abnormalities. Unusual placental abnormalities associated with perinatal complications also have been observed by ultrasonography such as placenta membranacea[60] or placenta percreta.[61]

Comment

Depending on the delay between the development of a lesion in utero and delivery, the ultrasonographic and pathologic findings can be very different. The ultrasonographic features of most placental vascular lesions may undergo major changes within few days. When placental abnormalities that could be associated with perinatal complications are suspected, serial ultrasonographic examinations should be performed.

Repeated ultrasonographic examination will also yield interesting pathophysiologic data on the development of placental lesions and may help in the prenatal differential diagnosis. For example, old thromboses are difficult to differentiate from infarcts if serial examinations are not performed. Repeated ultrasonographic examination is necessary to detect the evolution of a simple hypoechoic space into intervillous thrombosis or to differentiate a subamniotic cyst from a subamniotic hematoma. The increasing echogenicity of a chorioangioma with gestation can be related to fibrotic degeneration of a lesion,[27] which may indicate a reduced risk of high-output fetal cardiac failure.

Placental ultrasonographic examination must be combined with other prenatal investigations, some of which may help in the differential diagnosis. For example, elevated maternal serum levels of human chorionic gonadotropin are suggestive of trophoblastic disorders while elevated α-fetoprotein levels are less specific of a typical placental lesion[27, 62, 63] but indicate a breakdown of the maternal barrier such as may occur in infarcts or thrombosis.

Various vascular lesions have been found in placentas from pregnancies complicated by abnormal fetal or maternal Doppler indices.[64, 65] Although it is unlikely that a clinical decision will be made on placental morphologic features alone, the association of abnormal placental features with abnormal Doppler indices will clarify the hemodynamic changes occurring in these cases. Furthermore, the advent of color Doppler imaging may help to differentiate in utero between maternal and fetal vascular placental abnormalities.

REFERENCES

1. Hustin J, Schaaps JP. Echographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy. Am J Obstet Gynecol 1987; 157:162-8.
2. Schaaps JP, Hustin J. In vivo aspect of the maternal-trophoblastic border during the first trimester of gestation. Trophoblast Res 1988;3:39-48.
3. Hustin J, Schaaps JP. Anatomical studies of the uteroplacental vascularization in the first trimester of pregnancy. Trophoblast Res 1988;3:49-60.
4. Fisher CC, Garrett W, Kossoff G. Placenta aging monitored by gray scale echography. Am J Obstet Gynecol 1976;1:483-8.
5. Haney AF, Trought WS. The sonolucent placenta in high-risk obstetrics. Obstet Gynecol 1980;55:38-41.
6. Winberg F. Echogenic changes with placental aging. JCU 1973;1:52-5.
7. Grannum PAT, Berkowitz RL, Hobbins JC. The ultrasonic changes in the maturing placenta and their relation to fetal pulmonic maturity. Am J Obstet Gynecol 1979; 133:915-22.
8. Petrucha RA, Golde SH, Platt LD. Real-time ultrasound of the placenta in assessment of fetal pulmonic maturity. Am J Obstet Gynecol 1982;15:463-7.
9. Tabsh KMA. Correlation of real-time ultrasonic placental grading with amniotic fluid lecithin/sphingomyelin ratio. Am J Obstet Gynecol 1983;145:504-8.
10. Quinland RW, Cruz AC. Ultrasonic placental grading and fetal pulmonary maturity. Am J Obstet Gynecol 1982; 142:110-1.
11. Quinlan RW, Cruz AM, Buhi WC, Martin M. Changes in placental ultrasonic appearance: incidence of grade III changes in the placenta in correlation to fetal pulmonary maturity. Am J Obstet Gynecol 1982;144:468-70.
12. Quinlan RW, Cruz AC, Buhi WC, Martin M. Changes in placental ultrasonic appearance: pathologic significance of grade III placental changes. Am J Obstet Gynecol 1982;144:471-3.
13. Harman CR, Manning FA, Stearns E, Morrison I. The correlation of ultrasonic placental grading and fetal pulmonary maturation in five hundred sixty-three pregnancies. Am J Obstet Gynecol 1982;143:941-3.
14. Gast MG, Ott W. Failure of ultrasonic placental grading to predict severe respiratory distress in a neonate. Am J Obstet Gynecol 1983;146:464-7.
15. Kazzi GM, Gross TL, Sokol RJ, Kassi SNJ. Detection of intrauterine growth retardation: a new use for sonographic placental grading. Am J Obstet Gynecol 1983; 15:733-7.
16. Hills D, Irwin GAL, Tuck S, Baim R. Distribution of placental grades in high-risk gravidas. AJR 1984;143: 1011-3.
17. Ohel G, Granat M, Zeevi D, et al. Advanced ultrasonic placental maturation in twin pregnancies. Am J Obstet Gynecol 1987;156:76-8.
18. Proud J, Grant AM. Third trimester placental grading by ultrasonography as a test of fetal wellbeing. Br Med J 1987;294:1641-4.
19. Pinette MG, Loftus-Brault K, Nardi DA, Rodis JF. Maternal smoking and accelerated placental maturation. Obstet Gynecol 1989;73:379-82.
20. Fox H. Pathology of placenta. Philadelphia: WB Saunders, 1978.
21. Sands J, Dobbing J. Continuing growth and development of the third trimester placenta. Placenta 1985;6:13-22.
22. Vermeulen RCW, Lambalk NB, Exalto N, Arts NFT. An anatomic basic for ultrasound images of the human placenta. Am J Obstet Gynecol 1985;153:806-10.
23. Wilkin P. Pathologie du placenta. Paris: Masson, 1965.
24. Benirschke K, Driscoll SG. The pathology of the human placenta. New York: Springer-Verlag, 1967.
25. Jauniaux E, Avni FE, Donner C, Rodesch F, Wilkin P. Ultrasonographic diagnosis and morphological study of placentas circumvallate. JCU 1989;16:126-31.
26. Jauniaux E, Avni FE, Elkazen N, Wilkin P, Hustin J. Etude morphologique des anomalies placentaires echographiques de al deuxieme moitie de la gestation. J Gynecol Obstet Biol Reprod 1989;18:601-13.
27. Jauniaux E, Moscoso G, Campbell S, Gibb D, Driver M, Nicolaides KH. Correlation of ultrasound and pathologic findings of placental anomalies in pregnancies with elevated maternal serum alpha-fetoprotein. Eur J Obstet Gynecol 1990;37:219-30.
28. Kirkinen P, Jouppila P. Intrauterine membranous cyst: a report of antenatal diagnosis and obstetrics aspects in two cases. Obstet Gynecol 1986;67:26-30.
29. Spirt BA, Gordon L, Cohen WN, Yambao T. Antenatal

diagnosis of chorioangioma of the placenta. AJR 1980; 135:1273-5.
30. O'Malley BP, Toi A, deSa DJ, Williams GL. Ultrasound appearances of placental chorioangioma. Radiology 1981; 138:159-60.
31. Willard DA, Moeschler JB. Placenta chorioangioma: a rare cause of elevated amniotic fluid alpha-fetoprotein. J Ultrasound Med 1986;5:221-2.
32. Hoogland HJ. The ultrasound display of intervillous circulation. Placenta 1987;8:537-44.
33. Hoogland HJ, De Haan J, Vooys J. Ultrasonographic diagnosis of intervillous thrombosis related to Rh isoimmunization. Gynecol Obstet Invest 1979;10:237-45.
34. Spirt BA, Gordon LP, Kagan EH. Sonography of the placenta. In: Sanders I, Roger C, Everette JA, eds. The principles and practice of ultrasonography in obstetrics and gynecology. East Norwalk, Connecticut: Appleton-Century-Crofts 1985:333-53.
35. Szulman AE, Surti U. The syndromes of hydatidiform mole. I. Cytogenetic and morphologic correlations. Am J Obstet Gynecol 1978;131:665-71.
36. Szulman AE, Surti U. The syndromes of hydatidiform mole. II. Morphologic evolution of the complete and partial mole. Am J Obstet Gynecol 1978;132:22-7.
37. Kajii T, Ohama K. Androgenic origin of hydatidiform mole. Nature 1977;268:633-4.
38. Berkowitz R, Ozturk M, Goldstein D, Bernstein M, Hill L, Wands JR. Human chorionic gonadotropin and free subunits' serum levels in patients with partial and complete hydatidiform moles. Obstet Gynecol 1989;74: 212-5.
39. Fisher RA, Sheppard DM, Lawler SD. Twin pregnancy with complete hydatidiform mole (46,XX) and fetus (46,XY): genetic origin proved by analysis of chromosome polyumorphims. Br Med J 1982;284:1218-9.
40. Sande HA, Eyjolfsson O. Hydatidiform mole with a coexistent fetus. Acta Obstet Gynecol Scand 1985;64:353-5.
41. Szulman AE, Philippe E, Boue JG, Boue A. Human triploidy: association with partial hydatidiform moles and nonmolar conceptuses. Hum Pathol 1981;12:1016-21.
42. Doshi N, Surti U, Szulman AE. Morphologic anomalies in triploid liveborn fetuses. Hum Pathol 1983;14:716-23.
43. Block MF, Merrill JA. Hydatidiform mole with coexistent fetus. Obstet Gynecol 1982;60:129-34.
44. Broekhuizen FF, Eljade R, Hamilton PR. Early-onset preeclampsia, triploidy and fetal hydrops. J Reprod Med 1983;28:223-6.
45. Khoo SK, Monks PL, Davies NT. Hydatidiform mole coexisting with a live fetus: a dilemma of management. Aust N Z J Obstet Gynaecol 1986;26:129-35.
46. Camus M, Lefebvre G, Choukroun JB, Pons JC, Darbois Y. Triploidie et mole: a propos de deux cas evolutifs du deuxieme trimestre. J Gynecol Obstet Biol Reprod 1986; 15:315-20.
47. Rubenstein JB, Swayne LC, Dise CA, Gersen SL, Schwartz JR, Risk A. Placental changes in fetal triploidy syndrome. J Ultrasound Med 1986;5:545-50.
48. Meisner LF, Louie RR, Arya S, Gilbert EF. Triploidy with an extra sex chromosome (70,XXYY) and elevated alpha-fetoprotein levels. Birth Defects 1987;23:333-9.
49. Lockwood C, Scioscia M, Stiller R, Hobbins JC. Sonographic features of the triploid fetus. Am J Obstet Gynecol 1987;157:285-7.
50. Bendon RW, Siddiqi T, Soukup S, Srivastava A. Prenatal detection of triploidy. J Pediatr 1988;112:149-53.
51. O'Brien WF, Knuppel RA, Kousseff B, Sternlicht D, Nichols P. Elevated maternal serum alpha-fetoprotein in triploidy. Obstet Gynecol 1988;71:994-5.
52. Graham JM, Rawnsley EF, Simmons G, et al. Triploidy: pregnancy complications and clinical findings in seven cases. Prenat Diagn 1989;9:409-19.
53. Freeman SB, Priest JH, Macmahon WC, Fernhoff PM, Elsas LJ. Prenatal ascertainment of triploidy by maternal serum alpha-fetoprotein screening. Prenat Diagn 1989; 9:339-47.
54. Pircon RA, Towers CV, Porto M, Gocke SE, Garite TJ. Maternal serum alpha-fetoprotein and fetal triploidy. Prenat Diagn 1989;9:701-7.
55. Feinberg RF, Lockwood CJ, Salafia C, Hobbins JC. Sonographic diagnosis of a pregnancy with a diffuse hydatidiform mole and coexistent 46,XX fetus: a case report. Obstet Gynecol 1988;72:485-8.
56. Crooij MJ, Van Der Harten JJ, Puyenbroek JI, Van Geijn HP, Arts NFT. A partial hydatidiform mole, dispersed throughout the placenta, coexisting with a normal living fetus: a case report. Br J Obstet Gynaecol 1985;92: 104-6.
57. Buschi AJ, Brenbridge NAG, Cochrane JA, Teates CD. Hydropic degeneration of the placenta simultating hydatidiform mole. JCU 1979;7:60-1.
58. Sholl JS. Abruptio placentae: clinical management in nonacute cases. Am J Obstet Gynecol 1987;156:40-51.
59. Nyberg DA, Cyr DR, Mack LA, Wilson DA, Shuman WP. Sonographic spectrum of placental abruption. AJR 1987; 148:161-4.
60. Molloy CE, McDowell W, Armour T, Crawford W, Bernstine R. Ultrasound diagnosis of placenta membranecea in-utero. J Ultrasound Med 1983;2:377-9.
61. Cox SM, Carpenter RJ, Cotton DB. Placenta percreta: ultrasound diagnosis and conservative surgical management. Obstet Gynecol 1988;71:454-6.
62. Boyd PA, Keeling JW. Raised maternal serum alpha-fetoprotein in the absence of fetal abnormality: placental findings. Prenat Diagn 1986;6:369-73.
63. Salafia CM, Silberman L, Herrera NE, Mahoney MJ. Placental pathology at term associated with elevated midtrimester maternal serum α-fetoprotein concentration. Am J Obstet Gynecol 1988;158:1064-6.
64. Nessmann C, Huten Y, Uzan M. Placental correlations of abnormal umbilical Doppler index. Trophoblast Res 1988;3:309-24.
65. Jauniaux E, Campbell S. Fetal growth retardation with abnormal blood flows and placental sonographic lesions. JCU 1990;18:210-4.

Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes

Mark A. Plessinger, MS, and James R. Woods, Jr., MD
Rochester, New York

Cocaine produces a greater hypertensive response in pregnant ewes than in nonpregnant ewes. To determine whether pregnancy-related progesterone levels augmented this cocaine response, six nonpregnant ewes were surgically oophorectomized, implanted with femoral artery and vein catheters, and later administered progesterone 10 mg/kg intramuscularly for 3 days. Consistent responses in blood pressure and heart rate to intravenous norepinephrine 0.4 and 0.6 μg/kg, before and during progesterone treatment, indicated that progesterone did not increase α-adrenergic receptor sensitivity to exogenous norepinephrine. In contrast, intravenous cocaine at either 1.0 or 2.0 mg/kg produced approximately twofold greater increases in blood pressure and threefold greater increases in heart rate during each day of progesterone treatment when compared with responses to the same dose of cocaine before progesterone treatment. These results indicate that progesterone enhances the cardiovascular response to cocaine and does not involve an increase in α-adrenergic receptor sensitivity. (AM J OBSTET GYNECOL 1990;163:1659-64.)

Key words: Hormones, pregnancy, drugs of abuse, sheep, norepinephrine, cocaine

In a recent report we[1] demonstrated that cocaine, when given at the same dose per kilogram, produced a greater hypertensive response in pregnant ewes than in nonpregnant ewes. To explain these findings we hypothesized that pregnancy-related changes in hormonal levels may increase the cardiovascular sensitivity of pregnant ewes to cocaine. Since cocaine blocks the reuptake of catecholamines at nerve terminals, hormonally induced changes in α-adrenergic receptor sensitivity to norepinephrine may account for the increased cocaine sensitivity observed in pregnancy. Therefore the purpose of this study was to examine the heart rate and blood pressure responses to cocaine in oophorectomized nonpregnant ewes before and after treatment with the pregnancy hormone progesterone. Responses to norepinephrine before and during progesterone treatment also were examined to determine the influence of progesterone on α-adrenergic receptor sensitivity.

From the Department of Obstetrics and Gynecology, Strong Memorial Hospital, School of Medicine and Dentistry.
Supported by National Institutes of Health, National Institute on Drug Abuse grant No. 04415.
Presented in part at the Thirty-fourth Annual Meeting of the Society for Gynecologic Investigation, Atlanta, Georgia, March 18-21, 1987.
Received for publication May 8, 1990; revised June 28, 1990; accepted July 5, 1990.
Reprint requests: James R. Woods, Jr., MD, Strong Memorial Hospital, 601 Elmwood Ave., Box 668, Rochester, NY 14642.
6/1/23634

Material and methods

Six nonpregnant ewes were used in this study. Surgery, care, and use of sheep in this protocol have been approved by the University Committee on Animal Research of the University of Rochester to ensure compliance with the Animal Welfare Act of 1970 and the National Institutes of Health Policy on Laboratory Animals. Before surgery for implantation of catheters, each ewe was sedated with an intravenous injection of 0.02 mg/kg of a 100:1 ketamine hydrochloride/xylazine hydrochloride mixture preceding tracheal intubation. Anesthesia was maintained during surgery with halothane/oxygen by means of an Air-Shield Ventimeter ventilator 2 surgical respirator (Air-Shield, Hatsboro, Pa.). Catheters were placed in the femoral artery for measurement of arterial blood pressure and in the femoral vein for intravenous drug administration. Through a small abdominal incision, each ewe was oophorectomized to prevent hormonal cycling during the experimental period. The catheters were passed subcutaneously along the left flank and placed in a pouch secured to the ewe's side. Initial studies were conducted at least 5 days after surgery to allow for surgical recovery. Catheters were flushed daily with 1000 IU/ml heparin sodium.

Physiologic measurements. In each experiment blood pressure and heart rate were monitored continuously. Blood pressure was monitored by attaching the arterial catheter to a pressure transducer (Micron MP-

Table I. Baseline cardiovascular parameters before and during 3 days of progesterone treatment, 10 mg/kg intramuscularly, in six nonpregnant oophorectomized ewes

	Before progesterone	*Day of progesterone treatment*		
		1	*2*	*3*
Before norepinephrine injection				
Systolic blood pressure (mm Hg)	126 ± 7	124 ± 6	125 ± 4	127 ± 6
Diastolic blood pressure (mm Hg)	74 ± 8	71 ± 7	74 ± 5	74 ± 7
Mean arterial pressure (mm Hg)	91 ± 7	89 ± 7	91 ± 5	92 ± 6
Heart rate (beats/min)	90 ± 4	87 ± 2	89 ± 4	83 ± 4
Pulse pressure (mm Hg)	52 ± 5	54 ± 3	52 ± 3	53 ± 4
Before cocaine injection				
Systolic blood pressure (mm Hg)	126 ± 9	124 ± 7	123 ± 5	122 ± 5
Diastolic blood pressure (mm Hg)	71 ± 7	71 ± 7	69 ± 6	70 ± 6
Mean arterial pressure (mm Hg)	89 ± 7	89 ± 7	87 ± 6	87 ± 5
Heart rate (beats/min)	89 ± 5	91 ± 7	91 ± 6	88 ± 6
Pulse pressure (mm Hg)	55 ± 4	53 ± 2	54 ± 4	53 ± 4

Values are indicated for baseline before the first norepinephrine injection and before the first cocaine injection. Values represent mean ± SEM.

15D, Micron Instruments Inc., Los Angeles) that was connected to a pressure amplifier (SensorMedics 9853C, SensorMedics Corp., Anaheim, Calif.). Heart rate was measured by a cardiotachometer (SensorMedics 9857) connected in series with the blood pressure amplifier. Blood pressure and heart rate were continuously recorded on a SensorMedics R-611 eight-channel recorder. Pulse pressure was computed as systolic blood pressure minus diastolic blood pressure.

Doses

Norepinephrine administration. To evaluate α-adrenergic receptor sensitivity, 0.4 and 0.6 μg/kg norepinephrine [(−)arterenol bitartrate salt (Sigma Chemical Company, St. Louis)] in physiologic saline solution (0.9% sodium chloride) was given before and each day of progesterone administration. Norepinephrine 0.4 μg/kg was first administered intravenously as a 2 second bolus injection. At least 60 minutes passed before the 0.6 μg/kg norepinephrine dose was given in a similar manner. An additional 60-minute period elapsed after the 0.6 μg/kg norepinephrine dose before cocaine administration.

Cocaine administration. Doses of cocaine hydrochloride (Sigma) were dissolved in 5 ml of physiologic saline solution. Cocaine was administered intravenously as a 30-second bolus injection in a random fashion at either 1.0 or 2.0 mg/kg body weight. At least 2 hours was allowed to elapse before each animal received the second dose.

Progesterone administration. One to 3 days after the initial responses to norepinephrine and cocaine were obtained, each ewe was injected intramuscularly with progesterone (Eli Lilly & Co., Indianapolis) 10 mg/kg daily for three consecutive days. This dose level, when given to nonpregnant ewes, has been previously shown to produce serum progesterone levels comparable to those of pregnancy.[2] Approximately 2 hours after each progesterone administration, the two doses of norepinephrine and two doses of cocaine were injected as described above.

To determine progesterone levels, a 5 ml blood sample was drawn from the femoral vein catheter each day, approximately 2 hours after progesterone administration but before norepinephrine and cocaine administrations for later analysis of serum progesterone levels. These serum samples were analyzed for progesterone levels by solid-phase iodine 125 radioimmunoassay (Diagnostic Products Corporation, Los Angeles). The lowest level of detectability of this method is 0.05 ng/ml.

Data analysis. For data analysis baseline measurements of heart rate and blood pressure were obtained at 25 and 5 minutes before each respective drug administration and computed as a mean baseline value. Since most of the response activity to norepinephrine and cocaine occurred within the first 2 minutes, response measurements to norepinephrine administration were obtained at 10-second intervals over the first 2 minutes and at 5, 10, 15, and 30 minutes. Response measurements to cocaine were obtained at the same time intervals as the norepinephrine responses with an additional measurement at 60 minutes.

To determine statistical significance, a three-way [factors = dose (2), time (17 or 18), and day of progesterone treatment (4)] repeated measures analysis of variance with a post hoc Newman-Keuls range test was used to determine differences between means when a significant F ratio was obtained by analysis of variance. Statistical significance was established at a p value of <0.05.

Results

Administration of progesterone 10 mg/kg intramuscularly produced serum progesterone levels comparable to those observed during pregnancy in the

Table II. Maximum changes in cardiovascular parameters from baseline in response to 0.4 and 0.6 μg/kg norepinephrine before and during 3 days of progesterone treatment

		Day of progesterone treatment		
	Before progesterone	*1*	*2*	*3*
After 0.4 μg/kg norepinephrine injection				
Systolic blood pressure (mm Hg)	45 ± 4*	33 ± 13*	43 ± 7*	40 ± 8*
Diastolic blood pressure (mm Hg)	24 ± 3*	22 ± 7*	23 ± 3*	20 ± 5*
Mean arterial pressure (mm Hg)	31 ± 3*	25 ± 9*	29 ± 4*	27 ± 6*
Heart rate (beats/min)	−22 ± 5*	−11 ± 2	−18 ± 4	−12 ± 2
Pulse pressure (mm Hg)	21 ± 4*	11 ± 7*	20 ± 5*	19 ± 4*
After 0.6 μg/kg norepinephrine injection				
Systolic blood pressure (mm Hg)	55 ± 6*	50 ± 12*	49 ± 10*	44 ± 10*
Diastolic blood pressure (mm Hg)	27 ± 2*	27 ± 5*	27 ± 5*	28 ± 5*
Mean arterial pressure (mm Hg)	36 ± 3*	34 ± 8*	34 ± 7*	33 ± 7*
Heart rate (beats/min)	−22 ± 3*	−16 ± 2*	−19 ± 2*	−20 ± 4*
Pulse pressure (mm Hg)	28 ± 6*	23 ± 8*	23 ± 6*	16 ± 6

All maximum changes occurred at approximately 20 seconds after injection. Values represent mean ± SEM.
*Significant change from baseline value ($p < 0.05$).

Table III. Maximum changes in cardiovascular parameters from baseline in response to 1.0 and 2.0 mg/kg cocaine before and during 3 days of progesterone treatment

		Day of progesterone treatment		
	Before progesterone	*1*	*2*	*3*
After 1.0 mg/kg cocaine injection				
Systolic blood pressure (mm Hg)	22 ± 3*	69 ± 14*†	57 ± 6*†	56 ± 8*†
Diastolic blood pressure (mm Hg)	19 ± 3*	44 ± 5*†	43 ± 4*†	39 ± 5*†
Mean arterial pressure (mm Hg)	20 ± 3*	52 ± 8*†	48 ± 4*†	45 ± 6*†
Heart rate (beats/min)	−18 ± 5*	19 ± 21	38 ± 9*†	28 ± 9*†
Pulse pressure (mm Hg)	5 ± 1*	27 ± 8*†	15 ± 3*†	19 ± 6*†
After 2.0 mg/kg cocaine injection				
Systolic blood pressure (mm Hg)	44 ± 6*	72 ± 8*†	66 ± 12*	67 ± 6*†
Diastolic blood pressure (mm Hg)	39 ± 5*	54 ± 3*†	53 ± 7*	56 ± 4*†
Mean arterial pressure (mm Hg)	41 ± 6*	60 ± 4*†	57 ± 8*	59 ± 4*†
Heart rate (beats/min)	7 ± 4*	27 ± 12	45 ± 14*	31 ± 9*
Pulse pressure (mm Hg)	9 ± 2*	19 ± 6*	15 ± 7	15 ± 3*†

Values represent mean ± SEM.
*Significant change from baseline value ($p < 0.05$).
†Significant difference of progesterone treatment result as compared with before progesterone result ($p < 0.05$).

sheep.[2] The mean ± SEM progesterone levels in nanograms per milliliter serum were as follows: before progesterone, 0.28 ± 0.05; day 1 treatment, 97.8 ± 40.3; day 2 treatment, 125.3 ± 41.0; day 3 treatment, 119.4 ± 15.4. Baseline measurements of systolic pressure, diastolic pressure, mean arterial pressure, pulse pressure, and heart rate before norepinephrine and before cocaine administration during all 4 days of experimentation (before progesterone and during 3 days of progesterone treatment) demonstrated that progesterone treatment for 3 days had no significant effect on baseline cardiovascular values (Table I).

Norepinephrine produced dose-dependent and significant increases from baseline values in systolic blood pressure, diastolic blood pressure, mean arterial pressure, heart rate, and pulse pressure (Table II). For each dose of norepinephrine the maximum cardiovascular response was constant before and during all 3 days of progesterone treatment (Table II). However, the responses to cocaine demonstrated an approximately twofold greater and significant maximum increase from baseline values in systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure (Table III). In response to cocaine, 1.0 mg/kg, heart rate increased approximately threefold when compared with the heart rate response before progesterone was administered. Although a significant difference was not obtained at the 2.0 mg/kg dose, the trend toward increased heart rate response to cocaine after progesterone treatment was maintained (Table III).

The cardiovascular response values to norepinephrine and cocaine were plotted versus time for each of

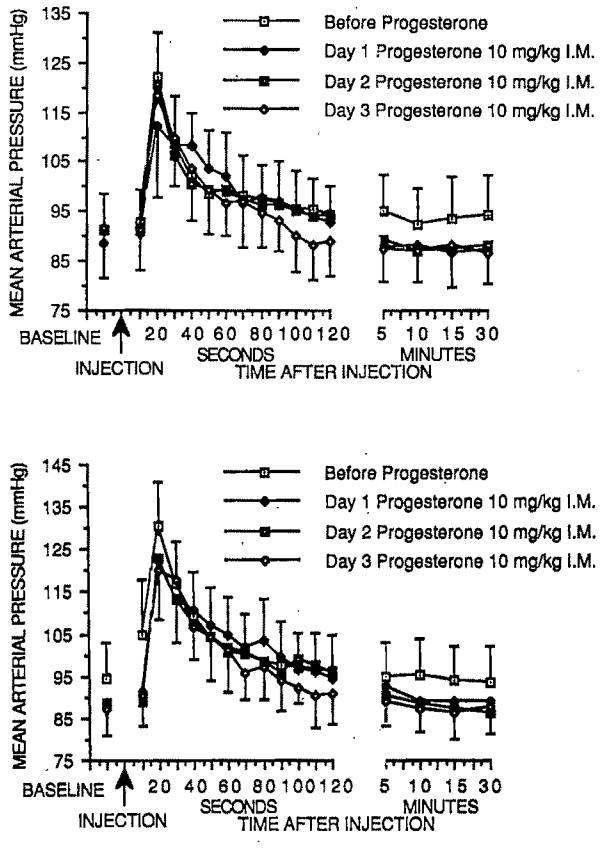


Fig. 1. Responses of mean arterial pressure to intravenous bolus injections of 0.4 μg/kg *(top)* and 0.6 μg/kg *(bottom)* norepinephrine plotted against time. Different *symbols* indicate responses before and during each of 3 days of progesterone treatment, 10 mg/kg intramuscularly. Values represent mean ± SEM from six oophorectomized ewes.

the 4 days of experimentation. For either dose of norepinephrine, the maximum response changes of mean arterial pressure occur at approximately 20 seconds after norepinephrine administration (Fig. 1). As illustrated, the mean arterial pressure responses to norepinephrine were constant over all 4 days of experimentation, indicating that progesterone treatment had no influence on the responses to norepinephrine. It is noteworthy that all responses to either dose of norepinephrine were virtually identical regardless of the day of experimentation or the timing sequence.

In contrast, when the cardiovascular responses to cocaine were plotted versus time, a different response pattern emerged. Cocaine produced its maximum effect on mean arterial pressure at approximately 40 seconds after injection (Fig. 2). Mean response values of mean arterial pressure were significantly increased after progesterone treatment from 40 to 120 seconds after cocaine injection but were equivalent to the response values to cocaine injection before progesterone treatment by 5 minutes and throughout the remainder of the experimental period. These results indicate that progesterone treatment augments the cardiovascular response to cocaine in nonpregnant ewes and that this augmented response occurs within the first 5 minutes after cocaine injection.

Comment

The results of this study indicate that treatment of nonpregnant oophorectomized ewes with the pregnancy hormone progesterone produced an increased cardiovascular responsiveness to intravenous cocaine. Cocaine produced an approximately twofold increase in systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure from their respective baseline values after treatment with 10 mg/kg progesterone intramuscularly when compared with the responses measured at the same dose of cocaine before progesterone treatment was initiated. In addition, the heart rate response to cocaine was increased approximately threefold after progesterone treatment. This increased responsiveness to cocaine occurred within hours after administration of the first dose of progesterone and was persistent over all 3 days with little difference between each day of progesterone treatment (Table III, Fig. 2). These results were consistent with our previous observation that pregnant ewes exhibit an increased cardiovascular responsiveness to cocaine when compared with responses from nonpregnant ewes given identical doses in milligrams per kilogram.[1] In addition, the cardiovascular responses of the progesterone-treated nonpregnant ewes to cocaine in the current study closely resemble those responses previously obtained from pregnant ewes.[1,3,4] We have hypothesized that increased sensitivity of pregnant ewes to cocaine may be due to an increased adrenergic receptor sensitivity, a change in cocaine metabolism, or an increased cardiac responsiveness, all of which may be dependent on an alteration in hormonal environment.

Cocaine exerts its pharmacologic action by blocking the uptake of released catecholamines within the synaptic cleft of nerve terminals, prolonging their actions at the effector terminals and increasing their concentrations in the bloodstream.[5] As a result, cocaine produces vasoconstriction by increasing the concentrations of norepinephrine locally in the synaptic cleft and systemically in the peripheral vasculature.

Exogenous norepinephrine was used in this study to determine whether progesterone treatment produced an alteration in α-adrenergic sensitivity. The lack of change in norepinephrine responses after progesterone when compared with the response before progesterone suggests that there is no increase in α-adrenergic receptor sensitivity that could account for the increased hypertension to cocaine observed in the progesterone-

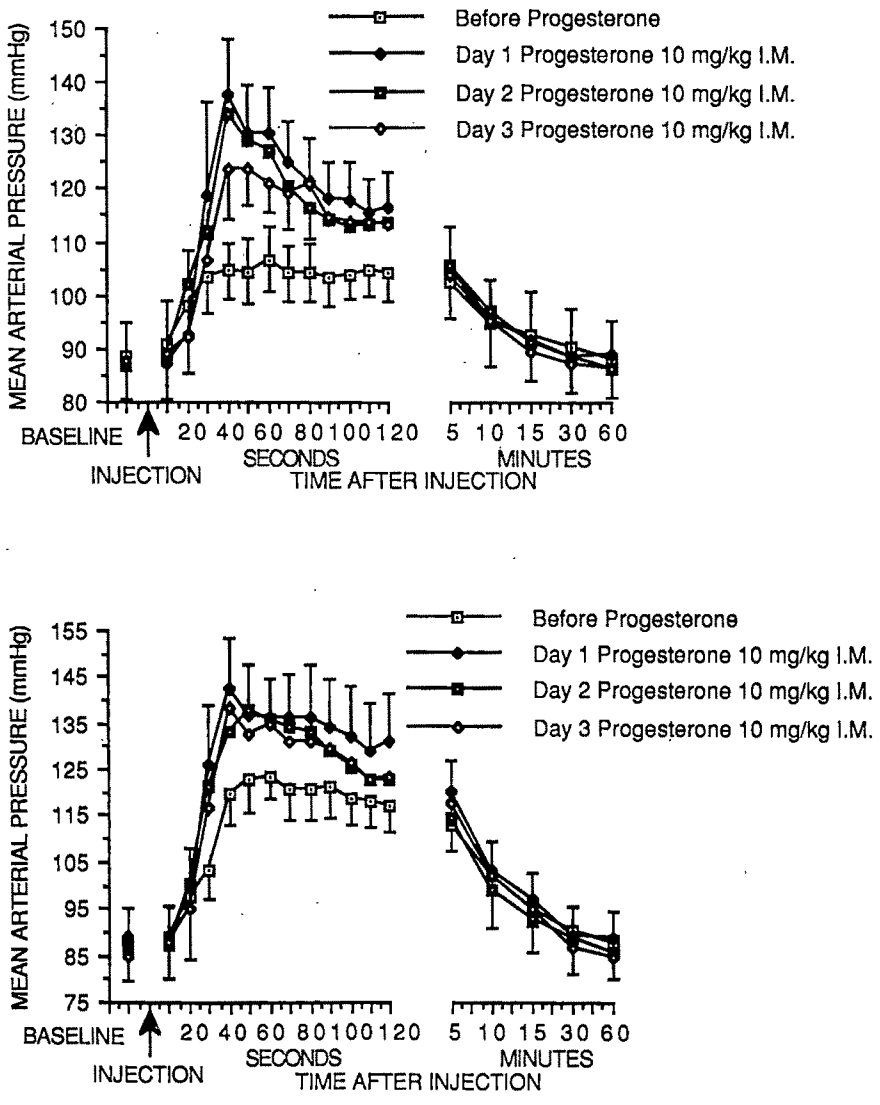


Fig. 2. Responses of mean arterial pressure to intravenous bolus injections of 1.0 mg/kg *(top)* and 2.0 mg/kg *(bottom)* cocaine. Different *symbols* indicate responses before and during each of 3 days of progesterone treatment, 10 mg/kg intramuscularly. Values represent mean ± SEM from six oophorectomized ewes.

treated ewes. Vascular α_1- and α_2-receptor activation by norepinephrine has been demonstrated to produce vasoconstriction and pressor responses in pithed dogs.[6,7] Because of these dual actions of norepinephrine, we cannot determine from our data whether progesterone increases the sensitivity of α_1- or α_2-subsites since norepinephrine is nonselective for these receptors. Progesterone could sensitize vascular α_1-receptors while desensitizing vascular α_2-receptors with the net effect producing no change in the pressor response to norepinephrine. Of note, the peak blood pressure responses to either dose of norepinephrine were higher before progesterone than after progesterone treatment (Fig. 1). If the progesterone-treated nonpregnant ewe is similar to the pregnant ewe in its cardiovascular sensitivity to norepinephrine, this trend is in keeping with observations by McLaughlin et al.[8] that the blood pressure response of pregnant ewes to intravenous norepinephrine is less than that of nonpregnant ewes.[8]

The increased cardiovascular responsiveness to cocaine after progesterone administration could be explained by an alteration in cocaine metabolism either as a decrease in the turnover rate of cocaine to its metabolic products or an increased turnover to bioactive metabolic products. Norcocaine is a reactive product of cocaine metabolism and is converted at a rate of 20% of the cocaine dose by *N*-demethylation.[9] Norcocaine is a potent vasoconstrictive agent that inhibits the reuptake of norepinephrine to a greater degree than cocaine.[10] The remaining cocaine and norcocaine

are metabolized by plasma and liver esterases and microsomal monooxygenases.[11] Reports in pregnant rats and rabbits have indicated a decrease in liver cytochrome P_{450} concentrations (−25%), as well as decreased specific activity of glucuronyl transferase[12] and monooxygenase[13] when compared with nonpregnant animals. Ochs et al.[14] demonstrated that progesterone treatment of nonpregnant rats increased the *N*-demethylation of aminopyrine, which has similar kinetics when compared with the *N*-demethylation of cocaine to norcocaine.[15] The progesterone treatment could alter the liver cytochrome P_{450} enzymes, glucuronyl transferases, and monooxygenases to decrease turnover of cocaine and/or increase the *N*-demethylation of cocaine to norcocaine. These changes in enzyme characteristics could produce a delay in the metabolism of cocaine or increase its conversion to norcocaine, explaining the increased responsiveness of the progesterone-treated nonpregnant ewes.

Progesterone could produce an increased cardiac responsiveness to cocaine. In the isolated heart muscle model, hydrocortisone treatment has been shown to increase the force of cardiac muscle contraction to cocaine.[16] This increase in cardiac contractility to cocaine, in the presence of this steroid in vitro, may explain the increased sensitivity of the progesterone-treated ewes by leading to an increased cardiac output and hypertension. The greater increase in systolic blood pressure and widened pulse pressure to cocaine seen after progesterone treatment may therefore reflect an increased cardiac contractility. Although cardiac output was not measured in the current study, cocaine may exert its drug action directly on the heart, producing a greater cardiac output after progesterone treatment. With heart rate used as a relative index of cardiac output, this is a reasonable conclusion since the heart rate response to cocaine was increased after progesterone treatment.

In summary, this study demonstrates that the pregnancy hormone progesterone increases cardiovascular sensitivity to cocaine when administered to nonpregnant ewes. This evidence explains in part the increased cardiovascular sensitivity to cocaine that we have previously observed in pregnant ewes when compared with nonpregnant ewes. Although the mechanism of increased sensitivity to cocaine in pregnant ewes is not clear, it appears to be at least partially related to a unique drug interaction between cocaine and progesterone. Since progesterone demonstrates an increase in cardiovascular sensitivity to cocaine, pregnant women or persons receiving progesterone supplementation may be at increased risk of cardiovascular complications during cocaine use.

REFERENCES

1. Woods JR Jr, Plessinger MA. Pregnancy increases cardiovascular toxicity to cocaine. AM J OBSTET GYNECOL 1990;162:529-33.
2. McLaughlin MK, Quinn P, Farnham JG. Vascular reactivity in the hind limb of the pregnant ewe. AM J OBSTET GYNECOL 1985;152:593-8.
3. Woods JR Jr, Plessinger MA, Clark KE. Effect of cocaine on uterine blood flow and fetal oxygenation. JAMA 1987;257:957-61.
4. Woods JR Jr, Plessinger MA, Scott K, Miller RK. Prenatal cocaine exposure to the fetus: a sheep model for cardiovascular evaluation. Ann N Y Acad Sci 1989;562:267-79.
5. Bayorh MA, Zukowska-Grojec Z, Kopin IJ. Effect of desipramine and cocaine on plasma norepinephrine and pressor responses to adrenergic stimulation in pithed rats. J Clin Pharmacol 1983;23:24-31.
6. Constantine JW, Gunnell D, Weeks RA. Alpha-1 and alpha-2 vascular adrenoceptors in the dog. Eur J Pharmacol 1980;66:281-6.
7. Constantine JW, Lebel W. Complete blockade by phenoxybenzamine of alpha-1 but not of alpha-2 vascular receptors in dogs and the effects of propranolol. Naunyn Schmiedebergs Arch Pharmacol 1980;314:149-56.
8. McLaughlin MK, Keve TM, Cooke R. Vascular catecholamine sensitivity during pregnancy in the ewe. AM J OBSTET GYNECOL 1989;160:47-53.
9. Just WW, Hoyer J. The local anesthetic potency of norcocaine, a metabolite of cocaine. Experientia (Basel) 1977;33:70-1.
10. Hawks RL, Kopin IJ, Colburn RW, Thoa NB. Norcocaine: a pharmacologically active metabolite of cocaine found in brain. Life Sci 1974;15:2189-95.
11. Stewart DJ, Inaba T, Lucassen M, Kalow W. Cocaine metabolism: cocaine and norcocaine hydrolysis by liver and serum esterases. Clin Pharmacol Ther 1979;25:464-8.
12. Neale MG, Parke DV. Effects of pregnancy on the metabolism of drugs in the rat and rabbit. Biochem Pharmacol 1973;22:1451-61.
13. Dean ME, Stock BH. Hepatic microsomal metabolism of drugs during pregnancy in the rat. Drug Metab Dispos 1975;3:325-31.
14. Ochs H, Dusterberg B, Schulte-Hermann R. Induction of monooxygenases and growth in rat liver by progesterone. Arch Toxicol 1986;59:146-9.
15. Stewart DJ, Inaba T, Kalow W. N-Demethylation of cocaine in the rat and in isolated rat hepatocytes: comparison with aminopyrine demethylation. J Pharmacol Exp Ther 1978;207:171-7.
16. Mugge A, Reupcke C, Schmitz W, Scholz H. Effects of (−)-phenylephrine on force of contraction in the presence of cocaine and hydrocortisone in cat papillary muscle. Naunyn Schmiedebergs Arch Pharmacol 1984;328:90-3.

Preferential mitogenic activity for myoblast-like cells can be extracted from uterine leiomyoma tissues

Michael Koutsilieris, MD, PhD, Josée Michaud, BSc, and Andreas Nikolis, BSc
Quebec, Quebec, Canada

The presence of mitogen(s) in leiomyoma extracts stimulating cells with the fibroblast, myoblast, and osteoblast phenotype was documented. Mitogenic activity in leiomyoma extracts was acid stable and sensitive to tryptic digestion. Reverse-phase high-performance liquid chromatography successfully separated mitogen(s) with preferential activity for myoblast cells from mitogens with a broad type of cell specificity and from inhibitors. This leiomyoma-derived preferential activity for myoblasts was absent in identically treated myometrial and endometrial extracts. This suggests that leiomyoma-derived substances with preferential growth factor activity for myoblast-like cells may play a role in the pathophysiologic characteristics of uterine leiomyomas. (Am J Obstet Gynecol 1990;163:1665-70.)

Key words: Growth substances, leiomyoma, inhibitors

Uterine leiomyomas are the most common tumors in adult women and one of the most common causes of infertility.[1] The use of electrophoretic types of X-linked enzyme glucose-6-phosphate dehydrogenase has indicated a unicellular histogenesis for leiomyomas.[2] Since fibrous tissue exhibited glucose-6-phosphate dehydrogenase heterogeneity, it was concluded that fibrous tissue within leiomyomas should originate from the same cells or cell clone from which muscle cells originate.[3] Recently a nuclear phosphoprotein (MyoDl) whose expression is restricted to proliferating myoblasts and differentiated myotubes was able to activate a myogenic program in fibroblasts, thus converting fibroblasts to myoblasts.[4] A short region of MyoDl complementary deoxyribonucleic acid (residues 143 to 162) was found to be similar to a conserved region in the c-*myc* family of proteins. Deletion of this region eliminated the capacity of the MyoDl protein to initiate myogenesis.[4] MyoDl phosphoprotein was able to initiate myogenesis in fibroblasts and, interestingly enough, in nonmesodermal cell lines.[4] This was suggestive of its role in explaining the unicellular origin of fibroblasts and myoblasts in uterine leiomyomas as previously reported.[3] The tendency of leiomyomas to develop and grow during the subject's reproductive life and to regress postmenopausally has implicated estrogens as a factor in their pathogenesis. This observation led to the hypothesis that women bearing such tumors would have either an increased serum level of estrogens or an increased concentration of estrogen receptors in the tumor. Since neither of these assumptions has been substantiated,[5-7] progestins, the biologic antagonists of estrogens, were implicated in the pathogenesis of leiomyomas.[8, 9] However, no conclusive data have been obtained.[8-12]

Recent reports have demonstrated differences between enzymatic activity of leiomyoma tissues and that of normal myometrium.[13, 14] Additionally human leiomyomas were found to contain significantly higher cytochrome P_{450} activity than adjacent normal myometrium.[15] Recently, changes in receptors for estradiol, progesterone, and epidermal growth factor in uterine fibromas were reported after treatment with luteinizing hormone–releasing hormone analogs.[16] In addition, the presence of luteinizing hormone–releasing hormone specific binding sites in uterine leiomyomas suggested a direct action of luteinizing hormone–releasing hormone analogs on this tissue.[17]

Previously we have reported the capacity of crude uterine extracts to stimulate cells with the osteoblast and fibroblast phenotype.[18] This suggested that uterine-derived growth substances may play a role in uterine pathophysiologic characteristics.[18, 19] This study characterizes certain uterine mitogens and inhibitors that act on myoblasts, fibroblasts, and osteoblasts. These substances were acid stable and tryptic sensitive. They could be retained and could be eluted from cartridges of C18 silica. Reverse-phase high-performance liquid chromatography (r-HPLC) identified, among various

From the Department of Obstetrics and Gynecology, Laval University, and the Endocrine Research Unit, Research Center, St-François d'Assise Hospital.
Supported by the Research Foundation, Hôpital St-François d'Assise, Laval University. M.K. is supported by a scholarship award (chercheur-boursier) from the "Fonds de la Recherche en Santé du Québec" (FRSQ).
Received for publication December 1, 1989; revised July 11, 1990; accepted July 16, 1990.
Reprint requests: Michael Koutsilieris, MD, PhD, Research Center, St-François d'Assise Hospital, Quebec, Quebec, Canada G1L 3L5.
6/1/23778

mitogens with broad-cell-type specificity, preferential mitogen(s) for myoblast-like cells in leiomyoma extracts. This mitogen(s) was absent from myometrial and endometrial extracts that were identically processed. These data suggest that inhibitory and proliferative substance(s) of leiomyoma extracts could formulate the humoral basis of the pathogenesis of uterine leiomyomas.

Material and methods

Tissue specimens were obtained during surgery on young, otherwise healthy women (aged 30 to 38; $N = 10$) who were diagnosed as having uterine leiomyomas and had not received any therapy. These patients had not received oral contraceptives during the last 6 months. Tissues were washed with Hanks balanced salt solution, pH 7.4, and kept frozen at −70° C. Representative tissue specimens underwent routine pathologic examination, and tissues from women in the proliferative ($n = 6$) and secretory ($n = 4$) phases were used in our studies. Tissue was first homogenized at 4° C in acetone (30 ml/gm). The insoluble precipitate was then homogenized in *n*-hexane (30 ml/gm), and the insoluble material from this procedure was homogenized again in acetone (30 ml/gm). The dried and defatted residue was then extracted individually in a mixture of 40 ml/gm of 1 mol/L hydrochloric acid containing 5% (vol/vol) formic acid, 1% (wt/vol) sodium chloride, and 1% (vol/vol) trifluoroacetic acid. This high acid content was to ensure that all peptidases were inhibited. Formic acid and sodium chloride ensured a high yield by minimizing binding of peptides to structural proteins.[18] This acidic extraction medium was designed to maximize solubilization of peptides while precipitating high-molecular-weight proteins. The protein concentration of the extracts was measured by the protein assay of Lowry et al.[20]

Fetal rat calvarial foreskin cells were used to assess the proliferative effects of leiomyoma extracts on fibroblasts.[18] Calvarial foreskins were incubated for 90 minutes at pH 7.4 in Hanks balanced salt solution containing 2 mg of crude collagenase per milliliter; 140 to 160 U/mg (Sigma Chemical Co., St. Louis). Cells released in Hanks balanced salt solution were grown in primary culture with RPMI 1640 medium (Gibco, Grand Island, N.Y.) containing 0% to 10% fetal calf serum (Gibco), depending on the experiment. The American Type Culture Collection mouse myoblast-like cell line (CRL-1456-G8) and the American Type Culture Collection rat smooth muscle embryonic thoracic aorta cell line (CRL-1476-A10) were used to assess the proliferative effect of leiomyoma extracts on myoblasts. The osteoblast-derived osteosarcoma cell line (UMR 106) was used to assess the proliferative effect of leiomyoma extracts on osteoblasts.[18, 19]

Cell lines were maintained in Dulbecco's modified medium F12 containing 10% fetal calf serum in 75 cm culture flasks. Cells were removed from the flasks with a mixture of 0.1% trypsin (Sigma) and 0.01% ethylenediaminetetraacetic acid (Sigma) in Hanks balanced salt solution and were precipitated with low-speed centrifugation. Cells were counted in a hematocytometer with trypan blue exclusion used to assess viability. Then cells were plated at 1.5×10^5 in 24-multiwell plates with Dulbecco's medium containing 0.5% fetal calf serum. Tissue culture was performed at 37° C in an atmosphere of 5% carbon dioxide and 95% air.[19]

To assess stimulation of deoxyribonucleic acid synthesis, samples of leiomyoma extracts or growth factor were added to cultures 48 hours after plating. Cultures were incubated with extracts for 22 hours and sequentially after 2 μCi of tritiated thymidine (specific activity 50 to 80 Ci/nmol; New England Nuclear, Boston) was added to each well. After 2 hours of incubation, the medium was aspirated and the cells were washed twice with ice-cold Hanks balanced salt solution and once with 1 ml of cold 1.2N perchloroacetic acid. The perchloroacetic acid–insoluble material was dissolved in 2 ml of 0.6N sodium hydroxide and tritiated radioactivity was measured by liquid scintillation spectrometry (counting efficiency = 50% to 60%).

The effects of fibroblast growth factor, epidermal growth factor, and insulin (all obtained from Sigma) on tritiated thymidine incorporation were assessed in all indicator cell culture systems.

Mitogenic activity of leiomyoma extracts (200 ng/ml; total protein concentration) was assessed before and after tryptic digestion with ×50 (wt/wt) crude trypsin (Sigma) at 22° C for 24 hours. Reaction was stopped by addition of aprotinin (Boehringer-Mannheim) (aprotinin/trypsin ratio = 15).

Samples of the leiomyoma extracts were first fractionated on cartridges of C18 Silica (Sep-Pak; Waters Associates, Milford, Mass.). Eluates from Sep-Pak testing by 80% acetonitrile containing 0.1% trifluoroacetic acid were lyophilized and then diluted by 0.1% trifluoroacetic acid and chromatographed on high-performance liquid chromatography. High-performance liquid chromatography was performed on a Pharmacia high-performance liquid chromatography system (Piscataway, N.J.) consisting of two LKB 2150 high-performance liquid chromatography pumps, one LKB 2152-020 controller, one LKB 2141 variable wavelength monitor, one LKB 2221 integrator, and one LKB 2212 Helirac fraction collector. Reverse-phase high-performance liquid chromatography was performed on a SuperPac Spherisord ODS2 No. 2134-256 LKB column. Linear gradients of acetonitrile containing 0.1% trifluoroacetic acid as a counter ion were performed over 60 minutes at a flow rate of 1.5 ml/min. Aliquots of the fraction eluted by reverse-

Table I. Example of effects of leiomyoma extracts on tritiated thymidine incorporation (percent above controls*)

Crude extract No. 1 (ng/ml)	*Fibroblasts*	*Myoblasts*	*Osteoblasts*
1	1 ± 5	2 ± 8	5 ± 4
100	28 ± 3	18 ± 4	20 ± 8
500	78 ± 8	35 ± 2	48 ± 9
1,000	96 ± 11	44 ± 8	101 ± 18
10,000	100 ± 14	50 ± 2	105 ± 15

*Percent above controls: 100 × Tritiated thymidine incorporation into cells incubated with extracts − Tritiated thymidine incorporation into cells incubated without extracts ÷ Tritiated thymidine incorporation into cells incubated without extracts. Control levels of tritiated thymidine incorporation into fibroblasts, myoblasts, and osteoblasts were 5833 ± 349, 3122 ± 121, and 15,644 ± 405 counts per minute, respectively. Each value expresses the mean and standard error of triplicate determinations.

Table II. Example of effects of tryptic digestion on mitogenic activity of 200 ng/ml crude leiomyoma extracts

	Tritiated thymidine incorporation (% above controls)*		
	Fibroblasts	*Myoblasts*	*Osteoblasts*
Before tryptic digestion	52 ± 9	27 ± 3	40 ± 4
After tryptic digestion	14 ± 5 (26.9%†)	5 ± 2 (18.5%)	11 ± 7 (27.5%)

*Percent above controls determined as described in footnote to Table I; control levels of tritiated thymidine incorporation into fibroblasts, myoblasts, and osteoblasts were 8332 ± 125, 6124 ± 740, and 18,222 ± 1011 counts per minute, respectively. Each value expresses the mean and standard error of triplicate determinations.

†Numbers in parentheses express the mitogenic activity as percent of its initial value.

Table III. Effect of known growth factors on tritiated thymidine incorporation (percent above controls*)

Growth factor (ng/ml)	*Fibroblasts*	*Myoblasts*	*Osteoblasts*
Fibroblast growth factor			
1	35 ± 5	20 ± 3	20 ± 4
10	79 ± 11	45 ± 8	58 ± 7
100	88 ± 5	87 ± 11	77 ± 8
Epidermal growth factor			
1	48 ± 6	5 ± 2	41 ± 4
10	69 ± 3	62 ± 4	100 ± 12
100	82 ± 6	133 ± 11	144 ± 9
Insulin			
500	20 ± 8	45 ± 5	30 ± 8
1,000	45 ± 3	154 ± 13	92 ± 14
10,000	88 ± 4	207 ± 28	185 ± 21
50,000	25 ± 8	145 ± 19	100 ± 17

*Percent above controls determined as described in footnote to Table I; control levels of tritiated thymidine incorporation into fibroblasts, myoblasts, and osteoblasts were 2213 ± 132, 1675 ± 27, and 22,090 ± 164 counts per minute, respectively. Each value expresses the mean and standard error of triplicate determinations.

phase high-performance liquid chromatography were tested for mitogenic activity on fibroblasts, myoblasts, and osteoblasts.

Results

Crude leiomyoma extracts were capable of stimulating tritiated thymidine incorporation into the deoxyribonucleic acid of cells with the fibroblast, myoblast, and osteoblast phenotype. The effect was dose-dependent (Table I).

Assessment of mitogenic activity of leiomyoma extracts before and after tryptic digestion of a given protein concentration (20 ng/ml) revealed a decrease in the activity of the trypsinized extracts by approximately 70% to 90% (Table II).

The response of our indicator cell cultures to fibroblast growth factor, epidermal growth factor, and insulin was documented. The effects of known growth factors on tritiated thymidine incorporation were dose-dependent (Table III). This indicated that cells could

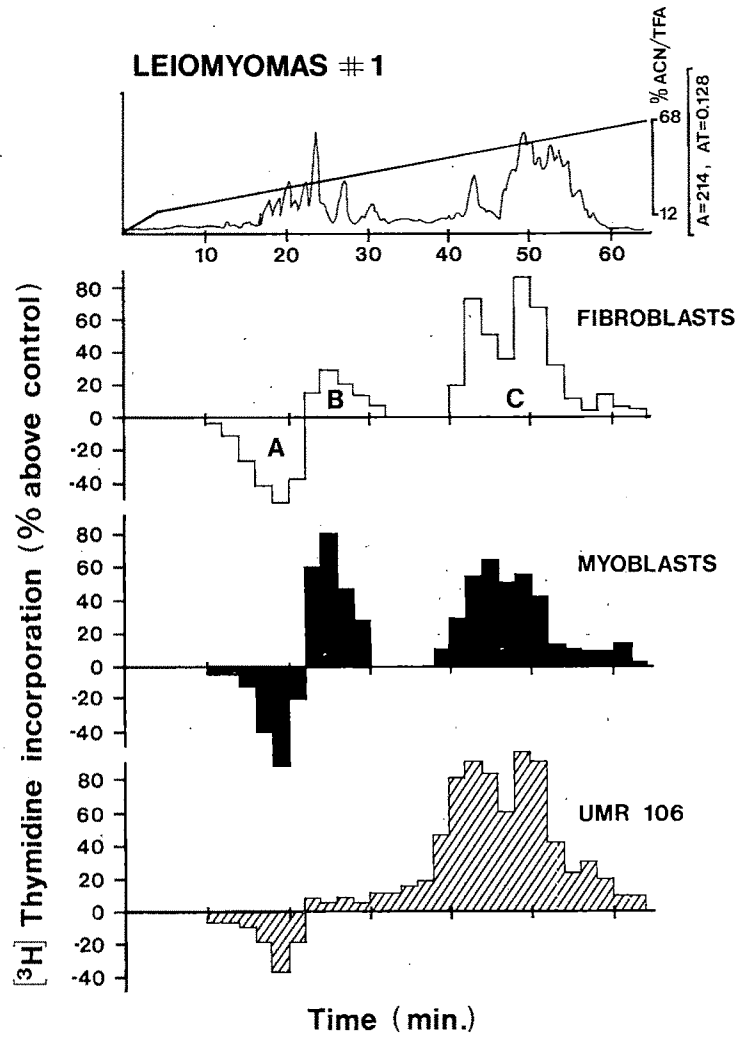


Fig. 1. An example of the ultraviolet profile of leiomyoma extracts (3.2 mg total protein concentration by assay of Lowry et al.[20]) fractionated on reverse-phase high-performance liquid chromatography column (LKB, SuperPac Spherisord ODS 2). Reverse-phase high-performance liquid chromatography was performed on an LKB system monitored for ultraviolet absorbance (-) and bioactivity in assays measuring tritiated thymidine incorporation into cellular deoxyribonucleic acid of fibroblast (□), myoblast (■), and osteoblast (▨) cells. Mitogenic activity was expressed as in footnote to Table I. The columns were eluted with linear gradients of acetonitrile *(ACN)* containing 0.1% trifluoroacetic acid *(TFA)*. Region *A* represents fractions exerting inhibitory activity for all cell types. Region *B* represents fraction with preferential mitogenic activity on myoblast cells. Region *C* represents fractions of reverse-phase high-performance liquid chromatography with mitogenic activity for all cell types.

respond to known mitogens. The response was of similar magnitude to that of leiomyoma extracts.

Reverse-phase high-performance liquid chromatography of leiomyoma extracts (n = 7; proliferative = 4; secretory = 3) revealed mitogenic activity in all three indicator cells (Fig. 1, region C). However, in one region, mitogenic activity specifically stimulated myoblasts, stimulated fibroblasts to a lesser degree, and had no effect on osteoblasts (Fig. 1, region B). This preferential mitogenic activity was detected in all tissue extracts but one (proliferative phase). In addition, one region with inhibitory activity for all three indicator cells was detected (Fig. 1, region A).

Reverse-phase high-performance liquid chromatography of extracts of myometrial (n = 11) and endometrial (n = 4) tissues, although processed identically and chromatographed under identical conditions, failed to reveal region(s) with preferential activity for myoblast cells (data not shown).

Comment

Although the effects of estrogens and progestins on the growth of uterine leiomyomas in vivo are unquestionable, the assumption that these hormones or their receptors are directly responsible for the development of the disease has not yet been substantiated. Also the

mechanism by which these hormones affect tumor growth is undetermined.[6-12]

Assessment of the mitotic activity in uterine leiomyomas revealed that the highest mitotic counts occurred during the early phase of the menstrual cycle, indicating indirectly that leiomyomal growth is affected by progesterone levels. In contrast, the number of and/or the regulation of cytoplasmic progesterone receptors present in normal tissue did not prove to be significantly different from that present in leiomyoma tissues.[8-12] Interestingly, cytoplasmic estradiol and progesterone receptor binding sites in leiomyoma tissues did not change during the menstrual cycle as they did in normal myometrium.[7]

Very recently, the presence of growth factors in normal uterine tissues was documented.[18] This growth substance(s) could stimulate (1) tritiated thymidine incorporation into cellular deoxyribonucleic acid and (2) cell number of cultures with the fibroblast and osteoblast phenotype. This effect was dose-dependent, and the presence or absence of fetal bovine serum in cell culture media did not alter the cellular response.[18, 19]

We extended this investigation to uterine leiomyoma extracts using the primary culture of fetal rat foreskin fibroblasts[18] and cells expressing the myoblast and osteoblast phenotypes as indicator cell systems.[19] The presence of mitogen(s) in crude leiomyoma extracts was steadly documented (Table I). A remarkable stimulation of tritiated thymidine incorporation in a dose-dependent manner similar to the one achieved by fibroblast growth factor, epidermal growth factor, and insulin was achieved (Tables I and III). The concentration range of insulin necessary to stimulate fibroblasts, myoblasts, and osteoblasts was indicative of an effect mediated by insulin-like growth factor type I receptors. The protein nature of mitogen(s) in leiomyoma extracts was evident since a remarkable decrease of the mitogenic activity was detected after tryptic digestion (Table II). In addition, the acid-stable character of this substance(s) was verified because mitogenic activity was extracted in a milieu of pH <2 (see extraction methods). These data suggested that fractionation of leiomyoma extracts with Sep-Pak cartridges of C18 silica was possible. Indeed mitogenic activity of leiomyoma extracts was retained and could be eluted by 80% acetonitrile over 0.1% trifluoroacetic acid from Sep-Pak cartridges (data not shown). These data lead us to the conclusion that reverse-phase high-performance liquid chromatography could be used for purification. Eluates from reverse-phase high-performance liquid chromatography were capable of stimulating tritiated thymidine incorporation into fibroblast, myoblast, and osteoblast-like cells (Fig. 1). Interestingly, one region with inhibitory activity for all indicator cells and another with preferential activity for cells with the myoblast phenotype were detected (Fig. 1). The above was a stable finding in all leiomyoma extracts tested except one. The region of preferential mitogenic activity for myoblasts was absent from all myometrial ($n = 11$) and endometrial ($n = 4$) extracts processed from adjacent uterine tissues and fractionated identically by reverse-phase high-performance liquid chromatography (data not shown).

The presence of mitogen(s) in leiomyoma extracts for fibroblast, myoblast, and osteoblast was expected although not reported previously elsewhere. Previous studies[18, 19] have detected similar mitogens in extracts from human uterine tissues. The presence of different growth factors such as fibroblast growth factor, epidermal growth factor, platelet-derived growth factor, and insulin-like growth factor type I in uterine extracts from different animals has been reported previously.[16] The presence of several growth substances in various normal and/or malignant tissue extracts is now also well documented. For all these growth substances different physiologic and pathologic roles have been assigned.[16, 19] In our opinion the most interesting finding of this study is the presence of preferential mitogenic activity for myoblasts extracted from leiomyoma tissues. The characterization and purification of such preferential mitogens deserves further attention. We believe that this growth substance(s) could be implicated in the pathophysiologic features of uterine leiomyomas and could mediate the action of estrogens and/or progestins on this tissue.

We thank our colleagues of the Departments of Obstetrics-Gynecology and Pathology of our hospital, namely, Drs. J. Belhumeur, J. Bergeron, J. Blanchet, J.-L. Dubé, J.-Y. Fontaine, C. Huot, R. Maheux, G. Quesnel, and M. Marois, for valuable support throughout the study.

REFERENCES

1. Parazzini F, La Vecchia C, Negri E, Cecchetti G, Fedele L. Epidemiologic characteristics of women with uterine fibroids: a case-control study. Obstet Gynecol 1988; 72:853.
2. Townsend DE, Sparkes RS, Baluda MC, McGlelland G. Unicellular histogenesis of uterine leiomyomas as determined by electrophoresis of glucose-6-phosphate dehydrogenase. Am J Obstet Gynecol 1970;107:1168.
3. Linder D, Gartler SM. Heterogeneity of fibrous tissue. Am J Hum Genet 1965;17:212.
4. Tapscott SJ, Davis RL, Thayer MJ, Cheng P-F, Weintraub H, Lassar AB. MyoDl: a nuclear phosphoprotein requiring a Myc homology region to convert fibroblasts to myoblasts. Science 1988;242:405.
5. Faber M, Conrad S, Heinrichs WL, Herrmann WL. Estradiol binding by fibroid tumors and normal myometrium. Obstet Gynecol 1972;40:479.
6. Punkka MJ, Kontula KK, Kauppila AJ, Jenne OA, Vihko RK. Estrogen receptor in human myoma tissue. Mol Cell Endocrinol 1976;6:35.
7. Eiletz J, Genz T, Pollow K, Schmidt-Gollwitzer M. Sex steroid levels in serum, myometrium and fibromyomata in correlation with cytoplasmic receptors and 17β-HSD activity in different age-groups and phases of menstrual cycle. Arch Gynecol 1980;229:13.
8. Büchi KA, Keller PJ. Cytoplasmic progestin receptors in

myomal and myometrial tissues. Acta Obstet Gynecol Scand 1983;62:487.
9. Pollow K, Geilfuss J, Boquoi E, Pollow B. Estrogen and progesterone binding protein in normal human myometrium and leiomyoma tissue. J Clin Chem Clin Biochem 1978;16:503.
10. Tamaya T, Fujumoto J, Okada H. Comparison of cellular levels of steroid receptors in uterine leiomyomata and myometrium. Acta Obstet Gynecol Scand 1985;64:307.
11. Pollow K, Sinnecter G, Boquoi E, Pollow B. In vitro conversion of estradiol-17β into estrone in normal human myometrium and leiomyomata. J Clin Chem Clin Biochem 1978;16:493.
12. Sales MR, McGarty KS. Leiomyomata: steroid receptor content variation with normal menstrual cycles. AM J OBSTET GYNECOL 1982;135:6.
13. Folkerd EJ, Newton CJ, Davidson K, Anderson MC, James VHT. Aromatase activity in uterine leiomyomata. J Steroid Biochem 1984;20:1195.
14. Herrmann U, Degiampietto P, Peheim E, Locher GW, Bachman C. Activities in leiomyomas and myometrium of enzymes involved in energy metabolism. Gynecol Obstet Invest 1987;73:194.
15. Senler TI, Jofmann GE, Sanfilippo JS, Barrows GH, Dean WL, Wittliff JL. Cytochrome P-450 activity in human leiomyoma and normal myometrium. AM J OBSTET GYNECOL 1985;153:551.
16. Lumdsden MA, West CP, Bramley TA, Rumgay L, Baird DT. The binding of epidermal growth factor to the human uterus and leiomyomata in women rendered hypoestrogenic by continuous administration of an LHRH agonist. Br J Obstet Gynaecol 1988;95:1299.
17. Wiznitzer A, Marbach M, Hazum E, Insler V, Sharoni Y, Levy J. Gonadotropin-releasing hormone specific binding sites in uterine leiomyomata. Biochem Biophys Res Commun 1988;152:1326.
18. Koutsilieris M. Human uterus-derived growth substances for rat bone cells and fibroblasts. AM J OBSTET GYNECOL 1989;161:1313.
19. Koutsilieris M, Michaud J. Characterization of the human uterus-derived growth substances [Abstract 634]. In: Proceedings of the annual meeting of the Endocrine Society, Atlanta, Georgia, June 20-23, 1990.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the Folin-phenol reagent. J Biol Chem 1951;193:265.

Platelet activating factor enhances in vitro fertilization of rabbit oocytes

William E. Roudebush, PhD, Brijinder S. Minhas, PhD, Deborah D. Ricker, MS, Thomas V. Palmer, BS, and Melvin G. Dodson, MD, PhD

Johnson City, Tennessee

Capacitation of spermatozoa is essential for fertilization. Rabbit spermatozoa are particularly difficult to capacitate in vitro and require treatment with high-ionic-strength Brackett's defined medium. Spermatozoa treated with platelet activating factor had significantly higher fertilization rates when compared with nontreated (fresh, twice washed) spermatozoa (63% vs 34%). Fertilization rates of spermatozoa treated with platelet activating factor, although higher than those of high-ionic-strength capacitated spermatozoa, were not significantly different (63% vs 57%). Spermatozoa treated with lyso–platelet activating factor, the biologically inactive form of platelet activating factor, were noted to have fertilization rates similar to those of the untreated (noncapacitated) group. These data show that synthetic platelet activating factor treatment of uncapacitated spermatozoa induces fertilization of rabbit oocytes in vitro in a manner similar to that for spermatozoa capacitated by high-ionic-strength media and significantly higher than that for untreated spermatozoa or after treatment with the biologically inactive form of platelet activating factor (lyso–platelet activating factor). (AM J OBSTET GYNECOL 1990;163:1670-3.)

Key words: Platelet activating factor, in vitro fertilization

From the Department of Obstetrics and Gynecology, James H. Quillen College of Medicine, East Tennessee State University.
Received for publication February 21, 1990; revised May 14, 1990; accepted July 17, 1990.
Reprint requests: Brijinder S. Minhas, PhD, Assistant Professor, Department of Obstetrics and Gynecology, James H. Quillen College of Medicine, East Tennessee State University, P.O. Box 19570A, Johnson City, TN 37614.
6/1/23860

Platelet activating factor (PAF) [1-0-alkyl-2-0-acetyl-*sn*-glycero-3-phosphocholine] is a potent phospholipid produced by many cell types including macrophages, neutrophils, and platelets and induces a variety of biologic and pharmacologic responses such as platelet aggregation, anaphylaxis, and vascular permeability.[1,2] PAF has also been reported to play a significant role in

reproduction.[3] Human and mouse preimplantation embryos produce and secrete PAF into culture medium in vitro,[4, 5] and embryo-derived PAF is associated with mild thrombocytopenia in early pregnancy.[6, 7] Furthermore, this initial maternal response of platelet activation occurred only in the presence of fertilized zygotes in mice. This mild thrombocytopenia was not observed during pseudopregnancy, in mice that have had tubal ligation and were mated to fertile male mice, or on the transfer of unfertilized eggs; these observations suggest PAF to be of embryonic origin. PAF administration to estrous mice and rabbits results in the appearance of early pregnancy factor in serum.[8, 9] PAF has been detected in rabbit and human ejaculated spermatozoa.[10, 11] However, PAF activity is not found in the seminal fluid.[11] PAF antagonists have been reported to exert spermicidal activity[12] both in vitro and in vivo after vaginal instillation. Synthetic PAF has been shown to have a stimulatory effect on human spermatozoa motility, and enhancement of the penetration rates of zona-free hamster oocytes with PAF-treated sperm has recently been reported.[13-15]

This study reports the effect of synthetic PAF on the in vitro fertilizing capability of rabbit spermatozoa. Fertilization rates of PAF-treated spermatozoa are compared with untreated (noncapacitated) fresh (twice-washed) spermatozoa, spermatozoa capacitated by exposure to high-ionic-strength Brackett's defined medium[16] and spermatozoa treated with a biologically inactive form of PAF (lyso-PAF).

Material and methods

Twenty-four New Zealand White rabbit does (3 to 4 kg) were primed with follicle-stimulating hormone (0.5 mg twice a day for 3 days; Schering, Kenilworth, N.J.) and human chorionic gonadotropin (hCG) (100 IU 12 hours after final dose of follicle-stimulating hormone; Sigma, St. Louis). Oocyte-cumulus complexes were collected 14 hours after hCG administration by flushing the oviducts with Ham's F-10 (Sigma) and treated with hyaluronidase (450 units/ml; Sigma) to remove cumulus cells. Three does did not respond to the simulation protocol. From 21 does, we obtained 407 oocytes (19.4 oocytes per doe), 391 of which were deemed to be morphologically normal. Mature oocytes were pooled, washed twice, and cultured in Brackett's defined medium[16] (300 mOsm/kg) until insemination.

Semen was collected from mature New Zealand White bucks ($n = 2$) (4 to 6 kg) by use of an artificial vagina and diluted with Brackett's defined medium to a final volume of 8.0 ml. Spermatozoa were washed twice by centrifugation (400 *g* for 5 minutes) in Brackett's defined medium and separated into control (fresh, twice-washed) and treatment groups (2 ml of spermatozoa suspension per treatment group). Spermatozoa in the three treatment groups were exposed for 15 minutes to (1) high-ionic-strength Brackett's defined medium (385 mOsm/kg) or (2) PAF (1×10^{-7} mol/L) (Bachem Bioscience, Philadelphia), or (3) lyso-PAF (1×10^{-7} mol/L) (Bachem Bioscience). PAF and lyso-PAF were stored in a stock solution of chloroform and methanol (1:4) before dehydration under a stream of nitrogen and rehydrated with Brackett's defined medium to the final concentration (1×10^{-7} mol/L) used. After the 15-minute exposure, each spermatozoa treatment group was washed twice (400 gm, 5 minutes) with 2.0 ml of Brackett's defined medium and immediately used for insemination. The mature (first polar body) oocytes were inseminated with 1×10^6 motile, fresh, high-ionic-strength Brackett's defined medium, PAF, or lyso-PAF–treated spermatozoa and cultured in Brackett's defined medium in an atmosphere of 5% carbon dioxide in air, 95% relative humidity at 39° C.

After 4 hours of coincubation, the oocytes were washed twice and cultured in Ham's F-10 medium (0.3% bovine serum albumin). Inseminated oocytes were examined for second polar body extrusion and/or formation of pronuclei 12 hours after insemination. The penetrated oocytes were cultured for another 12 hours for assessment of two-cell formation. Fertilization rates are reported as the number of oocytes that proceeded to the two-cell stage of development 24 hours after insemination.

Data were evaluated by a 2×2 χ^2 contingency table.

Table I. In vitro fertilization rates of cumulus-free ovulatory rabbit oocytes

Spermatozoa treatment	*No. of oocytes inseminated*	*Fertilization rate (2-cell formation)*
Control (fresh, washed twice)	101	35 (34.7%)*†
High-ionic-strength Brackett's defined medium	91	52 (57.1%)*
PAF	95	60 (63.2%)†‡
Lyso-PAF	104	45 (43.3%)‡

*$p < 0.01$.
†$p < 0.001$.
‡$p < 0.01$.

Results

PAF treatment of spermatozoa for 15 minutes resulted in a significant increase in the in vitro fertilization rate (63.2%, $p < 0.001$), compared with control untreated spermatozoa (34.7%) (see Table I). Capacitation of spermatozoa with high-ionic-strength treatment also resulted in significantly increased fertilization rates compared with controls (57.1% vs 34.7%, $p < 0.01$).

Fertilization rates with lyso-PAF (biologically inactive

PAF) were similar to the control rates and were significantly ($p < 0.01$) lower than the fertilization rates of the PAF-treated spermatozoa.

Comment

Freshly ejaculated spermatozoa must undergo a period of maturation in the female reproductive tract (capacitation) before they are capable of fertilizing an oocyte.[17] Soon after deposition in the female reproductive tract, spermatozoa undergo a filteration-like process by which they are separated from the seminal fluids, which contain capacitation inhibiting factors.[18] Using immunologic techniques, Oliphant and Brackett[19] demonstrated that in the rabbit system capacitation involves removal of seminal plasma components that coat the spermatozoal surface. Treatment of ejaculated rabbit spermatozoa with high-ionic-strength Brackett's defined medium capacitates rabbit spermatozoa in vitro and enhances the in vitro fertilization of oocytes, possibly by removing the plasma components coating the spermatozoa surface.[16]

In this study we noted the fertilization rates of cumulus-free rabbit oocytes to be similar with spermatozoa treated with PAF or high-ionic-strength Brackett's defined medium. PAF-treated spermatozoa fertilized significantly higher numbers of oocytes than twice-washed (control) and lyso-PAF–treated spermatozoa, indicating that PAF, by some yet unknown mechanism, induces the fertilizing capacity of spermatozoa, presumably by capacitation.

High-ionic-strength treatment of rabbit spermatozoa in our study has yielded fertilization rates of cumulus-free oocytes similar to those for in vivo capacitated spermatozoa.[20] PAF treatment of spermatozoa appears to capacitate rabbit spermatozoa as effectively as both the in vitro (high-ionic-strength Brackett's defined medium) and in vivo capacitation protocols.

The treatment of rabbit spermatozoa with CV3988, a PAF antagonist, before the artificial insemination of does has been reported to significantly decrease in vivo fertilization rates.[12] We have previously reported reductions in the in vitro fertilization rates of mouse oocytes in the presence of CV3988.[21] Furthermore, PAF treatment of mouse spermatozoa also has been shown to enhance in vitro fertilization rates of mouse oocytes.[22, 23]

Reduction of in vivo fertilization of rabbit oocytes on treatment of spermatozoa with PAF antagonists,[12] the presence of PAF in spermatozoa,[10] and enhancement of oocyte in vitro fertilization with treatment of spermatozoa with synthetic PAF are strong indicators of an important role for PAF in fertilization.

Previous reports from our laboratory have documented the presence of PAF in human spermatozoa,[11] stimulation of human spermatozoa motility,[13] and enhancement of in vitro fertilizability of mouse oocytes in the presence of PAF.[22] These data suggest a potential therapeutic use of PAF in promoting fertilization in vitro.

Further studies will be needed to evaluate the use of PAF in human fertilization in vitro and the potential therapeutic value for the treatment of male factor infertility and/or fertilization failure.

REFERENCES

1. Kumar R, Hanahan DJ. Diversity of the biochemical and biological behavior of platelet activating factor. In: Snyder F, ed. Platelet activating factor. New York: Plenum Press, 1987:29.
2. Braquet P, Rola-Pleszczynski M. Platelet activating factor and cellular immune responses. Immunol Today 1987; 8:345-52.
3. Harper MJK. Platelet activating factor: a paracrine factor in preimplantation stages of reproduction? Biol Reprod 1989;40:907-13.
4. Collier M, O'Neill C, Ammit AJ, Saunders DM. Biochemical and pharmacological characterization of human embryo-derived platelet activating factor. Hum Reprod 1988;3:993-8.
5. O'Neill C. Partial characterization of the embryo-derived platelet activating factor in mice. J Reprod Fertil 1985; 75:375-80.
6. O'Neill C. Examination of the causes of early pregnancy associated with thrombocytopenia in mice. J Reprod Fertil 1985;73:567-77.
7. O'Neill C, Gidley-Baird AA, Pike IL, Porter RN, Sinosich MJ, Saunders DM. Maternal blood platelet physiology and luteal phase endocrinology as a means of monitoring pre- and post-implantation embryo viability following in vitro fertilization. J In Vitro Fertil Embryo Transfer 1985;2:87-93.
8. Orozco C, Perkins T, Clarke FM. Platelet activating factor induces the expression of early pregnancy factor activity in female mice. J Reprod Fertil 1986;78:549-55.
9. Sueoka K, Dharmarajan AM, Miyazaki T, Atlas SJ, Wallach EE. Platelet activating factor–induced early pregnancy factor from the perfused rabbit ovary and oviduct. Am J Obstet Gynecol 1988;159;1580-4.
10. Kumar R, Harper MJK, Hanahan DJ. Occurrence of platelet activating factor in rabbit spermatozoa. Arch Biochem Biophys 1988;260:497-502.
11. Minhas BS, Kumar R, Dodson MG, Palmer TV, Harrill JL, Robertson JL. The presence of platelet activating factor-like activity in human spermatozoa and its implication concerning male infertility [Abstract 065]. In: Proceedings of the forty-fourth annual meeting of the American Fertility Society, Atlanta, Georgia, October 1988. Atlanta: American Fertility Society, 1988:S22.
12. Harper MJK, Woodard DS, Norris CJ. Spermicidal effects of antagonists of platelet activating factor. Fertil Steril 1989;51:890-5.
13. Ricker DD, Minhas BS, Kumar R, Robertson JL, Dodson MG. The effects of platelet activating factor on the motility of human spermatozoa. Fertil Steril 1989;52:655-8.
14. Minhas BS, Palmer TV, Roudebush WE, Fortunato SJ, Dodson MG. Platelet activating factor treatment of human spermatozoa enhances fertilization potential [Abstract 238]. In: Proceedings of the thirty-seventh annual meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990. St. Louis: Society for Gynecologic Investigation, 1990:215.
15. Angle MJ, Tom RA, McClure RD. The effect of platelet activating factor on sperm-egg interactions [Abstract 110]. In: Proceedings of the forty-fifth annual meeting of the

American Fertility Society, San Francisco, California, November 1989. San Francisco: American Fertility Society, 1989:S46.
16. Brackett BG, Oliphant G. Capacitation of rabbit spermatozoa in vitro. Biol Reprod 1975;12:260-74.
17. Austin CR. The capacitation of mammalian sperm. Nature 1952;170:326-7.
18. Overstreet JW. Transport of gametes in the reproductive tract of the female mammal. In: JF Hartman, ed. Mechanism and control of animal fertilization. New York: Academic Press, 1983:499.
19. Oliphant G, Brackett BG. Immunological assessment of surface changes of rabbit sperm undergoing capacitation. Biol Reprod 1973;9:404-14.
20. Seidel GE Jr, Bowen RA, Kane MT. In vitro fertilization culture and transfer of rabbit ova. Fertil Steril 1976; 27:861-70.
21. Minhas BS, Kumar R, Ricker DD, Randall GW, Dodson MG, Fortunato SJ. Inhibition of in vitro fertilization in mice by a platelet activating factor antagonist CV-3988 [Abstract 401]. In: Proceedings of the thirty-sixth annual meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989. San Diego: Society for Gynecologic Investigation, 1989:282.
22. Minhas BS, Kumar R, Ricker DD, Roudebush WE, Dodson MG, Fortunato SJ. The effects of platelet activating factor on mouse oocyte fertilization in vitro. AM J OBSTET GYNECOL 1989;161:1714-7.
23. Kuzan FB, Geissler FT, Henderson WR. Role of spermatozoal platelet activating factor in fertilization. Theriogenology 1989;31:214.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Adhesion formation after ovarian wound repair in New Zealand white rabbits: A comparison of ovarian microsurgical closure with ovarian nonclosure

Anne K. Wiskind, MD, Andrew A. Toledo, MD, A. Gatewood Dudley, MD, and Kathryn Zusmanis, BS

Atlanta, Georgia

Thirty female New Zealand white rabbits underwent standard laparotomy. Each ovary was bivalved and hemostasis was achieved with bipolar electrocautery. One ovary was then randomized to the closure group, whereas the other ovary was placed in the nonclosure group. In the closure group, the ovarian capsule was closed with a continuous suture of 8-0 Vicryl absorbable surgical suture material with microsurgical technique. In the nonclosure group, the ovaries were left open. Three weeks later the animals were killed and the ovarian adhesions were graded with a standardized scale by an observer (A. A. T.) blinded to the closure status of the animals. Five control animals underwent sham operations with minimal adhesion formation. Statistical analysis of the study animals by the paired Student *t* test showed a significantly higher adhesion score on the ovaries that were microsurgically closed compared with the ovaries not closed ($p = 0.02$). (Am J Obstet Gynecol 1990;163:1674-8.)

Key words: Ovarian wound, nonclosure, adhesion formation

Postoperative adhesion formation is one of the most unfortunate sequelae of gynecologic surgery and results in mechanical infertility, pelvic pain, and bowel obstruction. Adhesion formation after reconstructive ovarian surgery is particularly frustrating because it may have an important impact on future fertility. Adhesions coating the ovary may interfere with ovulation mechanics and distort tuboovarian relationships, thereby disrupting ovum transport.[1]

Many of the studies that have evaluated both the pathophysiology of adhesions and methods to deter their formation have involved the adhesiogenesis of peritoneal defects with relatively little attention focused on the ovary. However, procedures resulting in ovarian surgical wounds are commonly encountered in women of reproductive age who are then most likely to be adversely affected by the subsequent adhesions. Examples of such procedures include ovarian cystectomy, resection of endometriosis, wedge resection because of polycystic ovarian disease, and rarely, resection of an ovarian pregnancy.

The least adhesiogenic method of managing ovarian surgical defects remains to be determined. Previous studies showed that microsurgical closure with fine synthetic suture is superior to macrosurgical closure with chromic catgut suture.[2] Another study[3] showed that bipolar electrocautery of ovarian surgical defects is less adhesiogenic than macrosurgical closure. However, bipolar electrocautery of ovaries alone has been shown to cause significant adhesions.[3, 4] Thus far there has been no study that compares adhesion formation after ovarian closure using microsurgical technique with ovarian nonclosure. This was the purpose of our study.

Material and methods

The study was approved by the Institutional Animal Care and Use Committee of Emory University, PROTOCOL 125-89, and the animals were housed at Emory University under the supervision of university veterinary staff.

Thirty female New Zealand white rabbits of reproductive age that weighed 2500 to 4300 gm were anesthetized with ketamine hydrochloride 40 mg/kg and xylazine hydrochloride 10 mg/kg body weight. The abdomen was shaved and prepared with povidone-iodine solution, and the animal was draped with sterile sheets for abdominal surgery. Talc was carefully removed from the surgeon's gloves. The animals then underwent laparotomy through a midline abdominal incision approximately 4 to 5 cm long beginning just inferior to the umbilicus. The bowels were carefully packed in the upper abdomen with a moistened lap sponge and a pediatric Balfour retractor. With an op-

From the Department of Gynecology and Obstetrics, Emory University School of Medicine.
Funding was provided by a grant from the Emory Medical Care Foundation of Emory University.
Received for publication April 9, 1990; revised June 29, 1990; accepted July 17, 1990.
Reprint requests: Anne K. Wiskind, MD, Department of Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, GA 30303.
6/1/23863

erating microscope (model 1402, J.C. Hoppl Corp., distributed by Edward Weck Co., Research Triangle Park), the ovaries were each bivalved longitudinally to the level of the hilum with a No. 15 blade scalpel. Hemostasis of the ovary was achieved with bipolar electrocautery provided by a surgical cautery unit (Micro 120, Aspen Labs, Inc., Littleton, Colo.) on setting five.

Once this was completed on both ovaries, a card was pulled from a sealed envelope and one ovary was assigned to the closure group and the other ovary was assigned to the nonclosure group. In the closure group, the ovarian capsule was closed with a continuous, nonlocking suture of 8-0 Vicryl absorbable surgical suture material (Ethicon, Somerville, N.J.) on a V130-S tapercut needle. Care was taken to include just the ovarian capsule with minimal cortex in each bite of suture. The rabbit ovarian surface was quite friable, requiring that some cortex be included to achieve a smooth closure. The suture bites were no more than 1 mm in depth and were spaced about 1 mm apart for approximately seven to nine bites per ovary. In the nonclosure group the ovary was left open. The randomization of closure and nonclosure was equally distributed between the right and left ovaries.

Careful attention to meticulous microsurgical technique including gentle tissue handling, excellent hemostasis, and continuous irrigation with sterile saline solution was performed throughout the procedure. The Balfour retractor and laparotomy sponge were removed, the abdominal wall was closed with a continuous suture of 3-0 Vicryl absorbable surgical suture material, and the skin was closed with a subcuticular stitch of 4-0 Prolene sutures (Ethicon, Somerville, N.J.) reinforced by simple interrupted sutures of 4-0 Prolene. Descriptions of all operative procedures and notes of special problems were kept in a laboratory diary.

Five control animals also underwent laparotomy in the same fashion with visualization, mobilization, and handling of the ovaries with microforceps, but ovarian incisions, electrocautery, or sutures were not used.

All animals were allowed to recover routinely. Three weeks after the surgery, the animals were killed with a thiopental preparation (Pentothal) 60 mg/kg body weight administered intravenously. A second laparotomy was performed by an investigator blinded to the group status (A. A. T.) who had not been involved in any of the initial surgeries, and the pelvic adhesions were graded by a method suggested by Michael P. Diamond, MD (personal communication). A score of 0 to 1.0 was given for the percentage of ovarian surface involved with adhesions. Separate scores were given to the incision line, the lateral ovarian surface, and the medial ovarian surface, which were then added for a total adhesion score for each ovary (Table I, Fig. 1). Standard analysis of the data was performed with the paired Student *t* test.

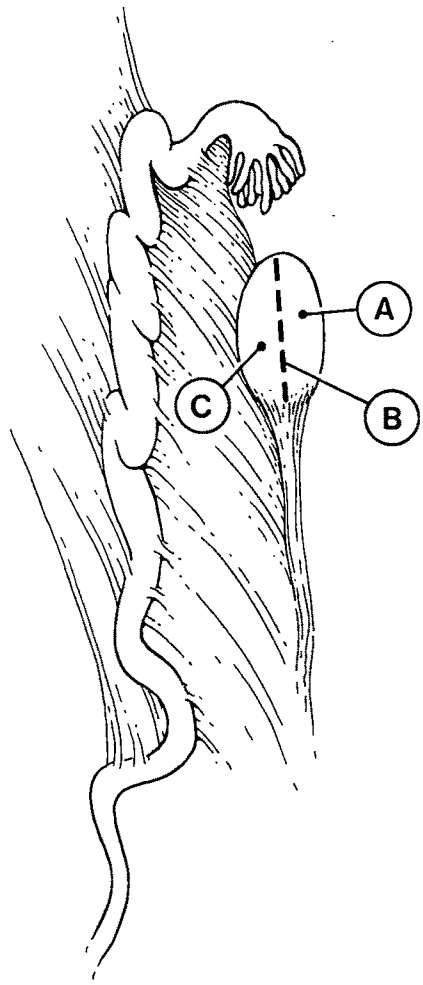


Fig. 1. Ovarian adhesion scale. *A*, Represents medial surface of rabbit ovary; *B*, incision line, and *C*, lateral surface. Percentage of each of these surfaces involved with adhesions is scored individually, then added for total adhesion score for ovary.

Table I. Adhesion score scale

Description of ovary	*Potential score*
Percentage of incision covered with adhesions	0-1.00
Percentage of lateral ovarian surface covered with adhesions	0-1.00
Percentage of medial ovarian surface covered by adhesions	0-1.00
Total adhesion score for each ovary	0-3.00

(Diamond MP. Unpublished data.)

Results

For statistical analysis the animals were divided into two groups: group A (ovarian microsurgical closure) and group B (ovarian nonclosure). Comparison of total

adhesion scores in group A (closure) and group B (nonclosure), summarized in Table II, shows a higher mean adhesion score in group A than that in group B, which is statistically significant to a p value of 0.02.

The five control animals were evaluated separately with no adhesion formation in three animals and minimal adhesions in the other two, suggesting that opening and closing the peritoneum alone contributed little to adhesion formation (Table III). There were also 14 ovaries (23%) in the study animals with an adhesion score of zero. Eight of these were in the nonclosure group, whereas six were in the closure group. Four animals (13%) had no adhesions on either ovary. Graphic plotting of the ovarian adhesion scores from each animal (Fig. 2) shows the higher adhesion scores in the ovarian closure group, with the exception of five animals in which ovarian nonclosure resulted in more adhesions that that of ovarian closure. After reviewing these cases it was found that in one animal the adhesions in both ovaries were minimal; however, the ovary that was closed underwent severe atrophy with subsequently fewer adhesions being formed than in the opposite normal-sized ovary that was not closed. One of the nonclosure ovaries in another animal was fixed deep in the pelvic sidewall. The mobilization necessary to position the ovary for bivalving and bipolar cautery was very difficult and traumatic. In fact, the ovary was separated into three pieces during the procedure, eliciting severe postoperative adhesion formation. In the remaining three animals, there was nothing unusual about the operations to indicate why nonclosure was more adhesiogenic than ovarian closure. It was the opinion of the statistician that these animals should be included in the study group, inasmuch as five of 30 were too many to exclude as outlying data points from a statistical standpoint. Furthermore, it was not considered statistically sound to exclude only one from this group even though excessive trauma was noted.

Marked atrophy was an interesting finding at second laparotomy in eight ovaries. These were evenly distributed among ovaries that were closed and those that were not closed. It was suspected to have occurred as a result of excessive bipolar cautery compromising the ovarian blood supply. However, it was also noted in cases in which only minimal cautery was used. The depth of the hilar incision was kept fairly consistent, but may be indirectly related to ovarian atrophy, inasmuch as the deeper the incision the more cautery required to achieve hemostasis.

In this study 11 ovaries were found encased in dense bowel adhesions. All but one of these ovaries had undergone microsurgical closure. The remaining ovary encased in dense adhesions was not closed, but had been mutilated by excessive trauma during the operative procedure.

Four animals had superficial wound separations. There was no evidence of postoperative evisceration or peritonitis in any of the animals, and they responded to treatment of local wound care. The operating time was approximately 1 hour for most animals, with the exception of the control animals that had an operating time of about 30 minutes.

Comment

In this study we showed that nonclosure of ovarian surgical defects is less adhesiogenic than microsurgical closure in the rabbit model. Most of the adhesions in the closure group involved the incision line; with 100% of the incision line involved in one third of the closed ovaries, and >50% of the incision line was involved in over half these ovaries. All but one case of bowel adhesions to the adnexa involved ovaries in the closure group. In contrast, the incision line was much less frequently involved in adhesions in the nonclosure ovaries. This is consistent with previous studies that showed suture to elicit a foreign body tissue reaction with subsequent adhesiogenesis.[5-7] The paucity of adhesions to the incision line in the nonclosure ovaries suggests that the cauterized ovarian parenchyma did not elicit a significant inflammatory response.

Trauma to tissue during the operative procedure also appeared to be a significant adhesiogenic factor. Most of these adhesions in the nonclosure ovaries involved the lateral and medial surfaces of the ovary and were most likely a result of trauma to the ovarian surface during manipulation. Instances in which excessive trauma to the tissue was noted during the operative procedure resulted in extensive postoperative adhesion formation. In fact, a partial explanation for the greater adhesions scores in the closure group may be the increased amount of manipulation required for surgical closure. Thus gentle, atraumatic tissue handling is of paramount importance in decreasing adhesion formation.[1, 7-10]

Preservation of the ovarian blood supply also is critical to ensure satisfactory results after ovarian reconstructive surgery. Compromise of the blood supply can result from certain types of surgical closure[5, 8] and from excessive electrocautery, as we suspect may have occurred in some of our animals with ovarian atrophy. Failure to preserve a functioning ovary negates the purpose of conservative ovarian surgery.

In applying this study in the rabbit model to the human patient, several factors must be considered. First, the rabbit ovary is much smaller and more friable than that in human beings, measuring approximately 0.8 × 0.5 × 0.3 cm, making subcapsular closure technically difficult, if not impossible, even with the operating microscope. A subcapsular closure that is possible on human ovaries may be less adhesiogenic than the

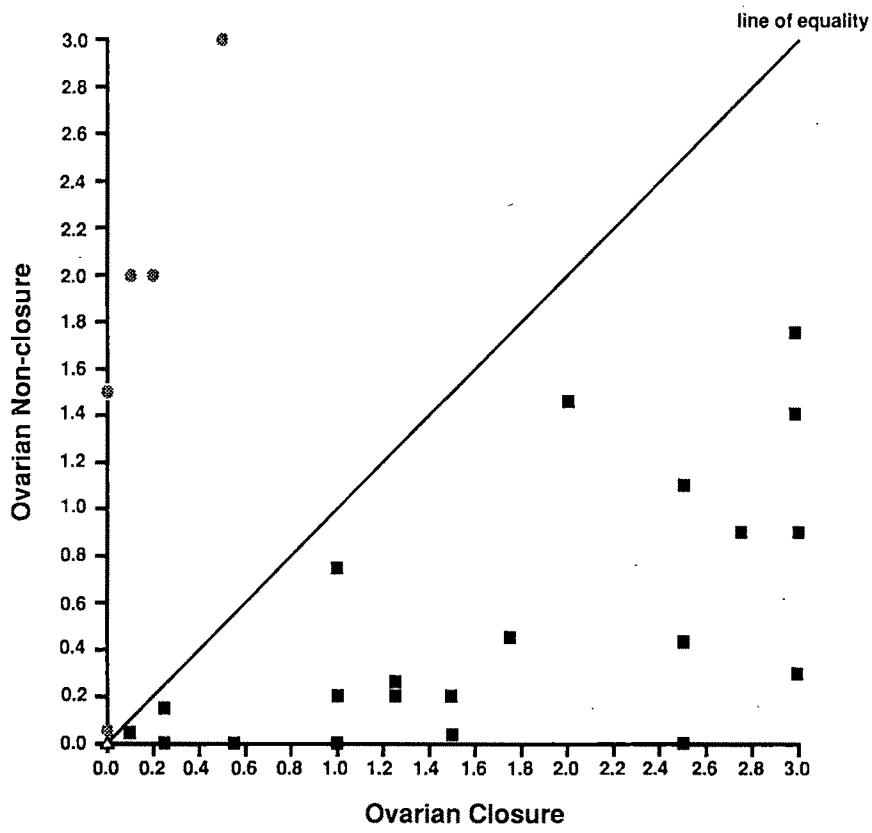


Fig. 2. Graphic representation of adhesion scores for each study animal. Δ Represents four animals with adhesion score of zero on both ovaries. ■ Represents 21 animals with adhesion score of microsurgically closed ovary greater than adhesion score of nonclosure ovaries. ● Illustrates five animals with nonclosure ovary adhesion score greater than adhesion score of ovary microsurgically closed.

continuous suture through the ovarian capsule used in this study.[5] Furthermore, after the surgical defects were created in the rabbit ovaries, the two halves of the ovaries naturally fell together, restoring their natural shape and appearance. In contrast, after many ovarian procedures in human beings, especially after resection of a cyst or an endometrioma, the remaining ovary is often a markedly distorted, thin, elongated shell. Whether adequate blood perfusion, hormone production, and tuboovarian relationships can be reestablished by this distorted ovary is yet to be determined.

In conclusion, our data show that ovarian microsurgical closure is significantly more adhesiogenic than ovarian nonclosure ($p = 0.02$). On the basis of these results, we suggest that for smaller surgical defects in which the ovarian architecture is not significantly altered, nonclosure of the defect is the management of choice. In larger defects in which the ovary is markedly distorted with a large denuded surface, the most appropriate management is unclear. At this time we recommend surgical reconstruction of the ovary by internal closure with a fine synthetic suture. With this technique the edges of the ovarian capsule can be reapproximated and left open. An additional layer of suture to close the ovarian surface is not only unnecessary, but more adhesiogenic. Special emphasis should be placed on atraumatic tissue handling to further minimize adhesion formation.

Table II. Adhesion scores in ovarian microsurgical closure (group A) versus ovarian nonclosure (group B)

	Mean adhesion score
Group A	1.21 (SD ± 1.12)
Group B	0.67 (SD ± 0.79)
Mean difference	0.543 (SD ± 1.25)

p Value = 0.02.

Table III. Adhesion scores in control animals

Control animal	*Adhesion score*
1	0
2	0
3	0.10
4	0.20
5	0

REFERENCES

1. Levinson CJ, Swolin K. Postoperative adhesions: etiology, prevention and therapy. Clin Obstet Gynecol 1980;23: 1213-20.
2. Eddy CA, Asch RH, Balmaceda JP. Pelvic adhesions following microsurgical and macrosurgical wedge resection of the ovaries. Fertil Steril 1980;33:557-61.
3. Rewis JR, Smith RP, Gallup DG. A comparison of severity of adhesion formation after two methods of ovarian wound repair in the rabbit model. In: Proceedings of the Society of Gynecologic Surgeons Meeting, New Orleans, Louisiana, March 1989.
4. Awadalla SG, Mattox JH, Slickenmyer WJ. The effect of cauterization of the rabbit model on adhesion formation. Fertil Steril 1986;46:696-700.
5. Oeslner G, Grabe RA, Boyers SP, Pan SB, Barnes ER, DeCherney AH. A comparison of three techniques for ovarian reconstruction. Am J Obstet Gynecol 1986; 154:569-72.
6. van Ryssel EJC, Brand R, Admiraal C, Smith I, Trimbos JB. Tissue reaction and surgical knots: the effect of suture size, knot configuration, and knot volume. Obstet Gynecol 1989;74:64-8.
7. Stangel JJ, Nisbet JD, Settles H. Formation and prevention of postoperative abdominal adhesions. J Reprod Med 1983;29:143-56.
8. Ellis H. The cause and prevention of postoperative intraperitoneal adhesions. Surg Gynecol Obstet 1971;133:497-511.
9. Jackson BB. Observations on intraperitoneal adhesions. Surgery 1958;44:507-14.
10. Buttram VC, Vaquero C. Post-ovarian wedge resection adhesive disease. Fertil Steril 1975;26:874-6.

Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis

Masahiro Sakata, MD,[a] Naoki Terakawa, MD,[a] Takahiro Mizutani, MD,[a] Osamu Tanizawa, MD,[a] Keishi Matsumoto, MD,[b] Nobuyuki Terada, MD,[c] and Katsuichi Sudo, PhD[d]

Osaka, Japan

Effects of danazol, gonadotropin-releasing hormone agonist (leuprolide), and danazol-leuprolide combination on experimental endometriosis were evaluated in female rats. A complete resorption of the fluid and a marked decrease in the volume of endometrium autotransplanted under the renal capsule were found after castration (1.4 ± 0.1 mm^3 in castrated, $n = 14$, vs 26.7 ± 5.6 mm^3 in intact control, $n = 16$). Histologic examination indicated atrophy and regression of the endometrial explant. These atrophic changes of endometrial explant were also induced by danazol, leuprolide, and the combination treatment. However, the volume of explants after combination therapy with danazol and leuprolide (1.8 ± 1.0 mm^3, $n = 17$) was significantly less than that after therapy with danazol (11.6 ± 2.8 mm^3, $n = 20$) or leuprolide alone (5.9 ± 1.4 mm^3, $n = 24$). The combination therapy (16/17) was also shown to be superior to danazol (9/20) or leuprolide alone (14/24) to induce the regression of fluid in experimental endometriosis. As expected, administration of leuprolide decreased the serum estradiol level, but use of danazol did not. These findings suggest that a combination therapy of danazol and gonadotropin-releasing hormone agonist, which show different modes of action, may be a potential modality in treatment of patients with advanced endometriosis. (Am J Obstet Gynecol 1990;163:1679-84.)

Key words: Endometriosis, danazol, gonadotropin-releasing hormone agonist, combination therapy

Endometriosis is the presence of functioning endometrial glands and stroma outside the uterine cavity. The prominent symptoms of the disease are chronic pelvic pain and infertility, which are significant problems in gynecology. The disease affects approximately 10% of all women of reproductive age, and the need for an effective nonsurgical treatment is pressing, particularly for patients concerned about their future fertility. Until recently, hormonal therapy of endometriosis has been dominated by use of danazol, an isoxazol derivative of 17α-ethinyltestosterone. The results of several studies have shown the efficacy of danazol in the treatment of endometriosis.[1] Dramatic improvement was noted in terms of both symptoms and signs and laparoscopic findings. The uncorrected fertility rate ranged between 41% and 50%. However, recurrences were common after completion of the treatment and the rates were more than 33% of treated patients.

The chronic administration of potent agonistic analogs of gonadotropin-releasing hormone (GnRH) suppresses gonadal steroid production. Recently GnRH agonist has been used as a form of hormonal treatment for sex steroid–dependent diseases such as endometriosis. In fact, the clinical usefulness of GnRH agonists in the treatment of endometriosis was demonstrated.[2] On the basis of the result of a multicenter double-blind comparative clinical study,[3] both danazol and GnRH agonist nafarelin therapy remarkably reduced the extent of disease as assessed by laparoscopy, and there were no statistically significant differences in the improvement of laparoscopic scores between the two regimens. Of the patients who attempted pregnancy, 39% succeeded after the completion of treatment; similar rates of pregnancy were obtained by both treatments. These results demonstrate that GnRH agonist is as effective as danazol in the treatment of endometriosis. However, a clinical study[3] also showed that the treatment with either danazol or GnRH agonist resulted in a complete resolution in only a few patients with advanced stages. Therefore the establishment of more

From the Departments of Obstetrics and Gynecology[a] and Pathology,[b] Osaka University Medical School, the Department of Pathology, The Center for Adult Diseases,[c] and Biology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.[d]
Supported in part by grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture in Japan.
Received for publication February 15, 1990; revised June 8, 1990; accepted June 29, 1990.
Reprint requests: Naoki Terakawa, MD, PhD, Department of Obstetrics and Gynecology, Tottori University School of Medicine, Nishimachi, Yonago 683, Japan.
6/1/23592

effective hormonal therapy in the treatment of patients with advanced endometriosis is necessary.

The most probable mechanism of action of GnRH agonist–induced resorption of endometriosis is estrogen deprivation and subsequent atrophy.[2-4] Conversely, the mode of action of danazol on the disease is not fully understood. It has been suggested that the action of danazol on endometriosis is attributable to the suppression of ovarian function, caused by either its antigonadotropic nature or inhibition of ovarian steroidogenic enzymes.[1] However, the pharmacologic action of danazol seems to be complex. During danazol treatment serum concentration of sex hormone–binding globulin was markedly suppressed, which resulted in a significant elevation of free testosterone.[5] Danazol also binds to multiple classes of steroid receptors and behaves as an androgen, glucocorticoid, or progestin.[1] A clinical study demonstrated that danazol binds to progestin receptors and shows progestin activity in human endometrium, suggesting a local progestin activity of the drug.[6] Furthermore, a short-term treatment with danazol induced atrophic change in the endometrium without a decrease in the level of serum estradiol.[7] An inhibitory effect of danazol on the growth of human endometrial cells[8] and endometrial adenocarcinoma cells[9] in culture was also demonstrated. These results suggest that danazol acts directly on the endometriotic tissues, and the action mechanism of danazol on endometriosis is different from that of GnRH agonist. A combined therapy with danazol and GnRH agonist may obtain a greater response rate than danazol or GnRH agonist alone in the treatment of patients with endometriosis. Therefore we initiated a trial of experimental endometriosis in female rats to determine whether a combined treatment with danazol and GnRH agonist is superior to danazol or GnRH agonist alone. In addition, possible mechanisms of action of these compounds in endometriosis are discussed.

Material and methods

Study design. To evaluate the effects of compounds precisely, experimental endometriosis was induced in 9-week-old Sprague-Dawley female rats by autotransplantation of endometrium under the renal capsule. After laparotomy the left uterine horn was resected and was opened by longitudinal incision, and the endometrium was dissected from the myometrium. Then a 5 × 5 mm section of endometrium was grafted under the capsule of left kidney of the same animal. Two weeks later the attachment and the viability of endometrial explant were examined by second laparotomy, and the size (length, width, and height) of the explant was measured and the volume was then calculated. The criterion for determination of a viable graft was fluid accumulation. The rate of induction of endometriosis was found to be >80%. The animals with endometriosis (with weights of approximately 250 gm) were randomly divided into five groups: intact rats treated with (1) vehicle alone (intact control), (2) GnRH agonist leuprolide depot formulation (1 mg/kg body weight), (3) danazol (100 mg/kg body weight), (4) or danazol-leuprolide combination, and (5) ovariectomized rats treated with vehicle alone. Daily administration of danazol or vehicle was given by gastric gavage for 3 weeks. Leuprolide depot (leuprolide) was injected once subcutaneously.

In the preliminary study the doses of two agents in female rats (1 mg/kg body weight for leuprolide and 100 mg/kg body weight for danazol) were found to be optimal because the serum concentrations of leuprolide and danazol measured after treatment for 3 weeks were in the same range of those obtained in the clinical use. At third laparotomy performed 3 weeks after the beginning of treatment (24 hours after the last administration of danazol or vehicle), the effects of the treatments on endometriosis were evaluated. The presence of fluid accumulation and the size of endometrial explant were examined. Then the explant was excised and fixed in 10% formalin for histologic evaluation. The ovaries, uterus, and pituitary gland were removed and weighed. Pituitary glands were immediately stored at −80° C for GnRH binding assay. Blood was drawn from the descending aorta, centrifuged, and serum was stored at −20° C for hormone assays. The guidelines for the care and use of the animals in this study were approved by Osaka University Medical School.

Hormone assays. Serum concentrations of luteinizing hormone, follicle-stimulating hormone, and estradiol were measured by radioimmunoassay as described previously.[10] The minimum detectable level of assay was 5 pg/tube for NIADDK-rLH RP-2,* 20 pg/tube for NIADDK-rFSH RP-2,* or 3 pg/tube for estradiol. The intraassay coefficients of variation in these assays were 8.5% for luteinizing hormone, 9.0% for follicle-stimulating hormone, and 8.0% for estradiol. Serum concentrations of danazol and leuprolide were also determined with high-performance liquid chromatography and radioimmunoassay as reported previously.[10, 11] These assays were performed by courtesy of the Division of Research and Development, Tokyo Tanabe Co., Ltd. (Tokyo), and Central Research Division, Takeda Chemical Industries, Ltd. (Osaka). The assay sensitivity was 3 ng/ml for danazol and 5 pg/tube for leuprolide.

GnRH receptor assays. Iodination of GnRH agonist leuprolide and measurement of pituitary GnRH receptors were performed as reported previously[12] unless specified otherwise. Estimation of GnRH receptors was

*Second international reference preparation from the National Institute of Arthritis, Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

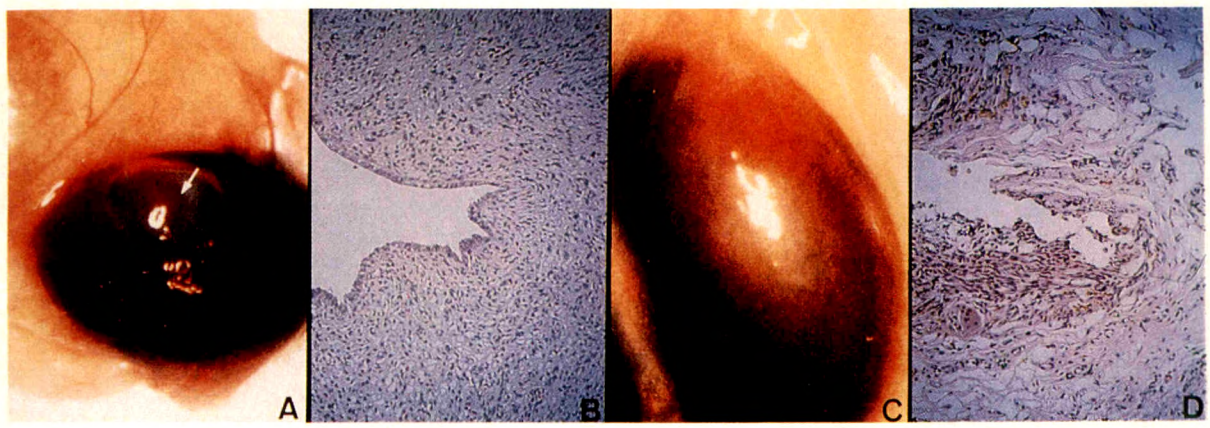


Fig. 1. Gross and microscopic appearance of experimental endometriosis at third laparotomy in intact control and ovariectomized rats. In intact control, fluid accumulates under the left renal capsule and an endometrial explant *(arrow)* is proliferative and well developed **(A)**. Histologically, both gland and stroma of the explant are well preserved **(B)**. In ovariectomized rat no fluid is found and the explant is visually absent **(C)**. Histologically, marked atrophy and regression of the explant are shown **(D)**. (Hematoxylin and eosin ×80.)

done with membrane preparations from a single pituitary by the incubation at 4° C for 4 hours. Because the affinity for specific GnRH binding sites in pituitaries of female rats was shown to be unchanged by danazol or leuprolide treatment, a saturating dose (1.25 nmol/L) of iodine 125 leuprolide, with or without 1 μmol/L of unlabeled leuprolide, was used to determine the binding sites in this study. GnRH binding sites were expressed as femtomoles per milligram of protein of pituitary gland.

Statistics. All values were expressed as mean ± SE, and analysis of variance was used for the statistical analysis. The evaluation of treatment on the regression of fluid in experimental endometriosis was analyzed statistically with the χ^2 test.

Results

All endometrial explants in 16 intact control animals were maintained essentially in the same size throughout the study (5.1 ± 0.7 mm in length × 4.4 ± 0.5 mm in width × 2.0 ± 0.2 mm in height at second laparotomy vs 4.8 ± 0.6 mm × 4.2 ± 0.5 mm × 2.0 ± 0.2 mm at third laparotomy). The gross and microscopic appearance of an explant at third laparotomy in intact control is shown in Fig. 1, *A* and *B*. Fluid accumulates under the renal capsule and the endometrial explant is proliferative and well developed. Histologically both gland and stroma of the explant are well preserved. Conversely, a complete resorption of the fluid and a marked decrease in size of the explant occurred after castration. Gross and microscopic appearance of an explant in ovariectomized rat is shown in Fig. 1, *C* and *D*. The explant is visually absent. Histologic examination also shows marked atrophy and regression of the explant.

The findings obtained in intact control and ovariectomized animals indicated that endometrial explant that is proliferating maintains essentially the same volume and shows accumulated fluid. Explant that became atrophic markedly reduced the volume and lost fluid. Therefore, in this study, the effectiveness of treatment on experimental endometriosis was evaluated with two separate parameters: the volume of the endometrial explant and formation of the fluid. At second laparotomy the rats with endometriosis was randomly divided to five groups of intact control, castration, danazol treatment, leuprolide treatment, and danazol and leuprolide combination. The volume of explants (mean ± SE) was 30.0 ± 6.2, 29.9 ± 6.8, 28.0 ± 4.8, 27.1 ± 3.8, and 27.9 ± 5.4 mm^3, respectively. The volume of explant in each individual at third laparotomy and the means of the volumes for each group are shown in Fig. 2. Although the volumes of endometrial explants in intact control rats ranged widely from 8.3 to 86.0 mm^3 (26.7 ± 5.6 mm^3), all the explants maintained essentially the same volume throughout the experiment. Endometrial explants markedly reduced to 1.4 ± 0.1 mm^3 ($p < 0.01$) in ovariectomized rats. The finding in ovariectomized animals shows that estrogen deprivation induces a marked regression of the experimental endometriosis. Endometrial explants also decreased to 11.6 ± 2.8 mm^3 ($p < 0.05$) in danazol-treated rats and to 5.9 ± 1.4 mm^3 ($p < 0.01$) in leuprolide-treated animals. It was interesting to find that the explants markedly reduced to 1.8 ± 1.0 mm^3 ($p < 0.01$) in danazol-leuprolide combination treatment. The volume of explants after the combination treatment was significantly less than that after danazol treatment ($p < 0.01$) or leuprolide treatment alone ($p < 0.05$) (Fig. 2).

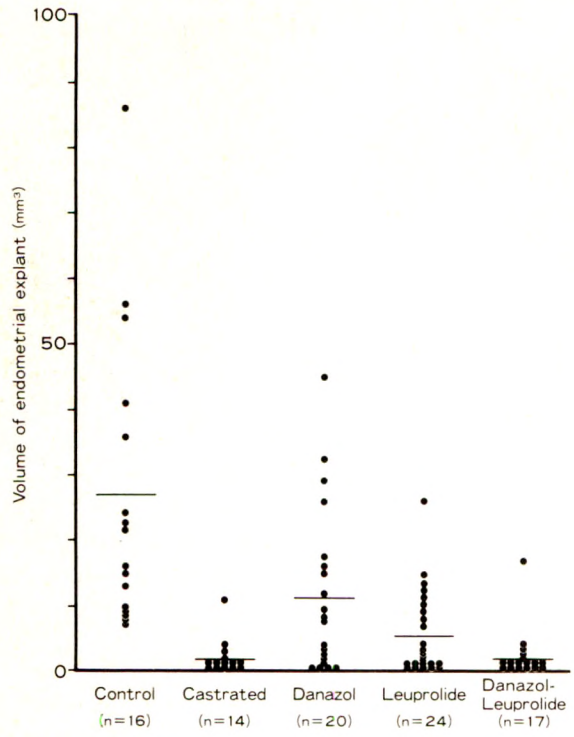


Fig. 2. Therapeutic effects of ovariectomy, treatment with danazol, leuprolide, or danazol-leuprolide combination on experimental endometriosis in female rats. The volume of endometrial explant (in cubic millimeters) in each individual at third laparotomy and the means of the volumes for each group *(solid line)* are shown. Danazol (100 mg/kg body weight per day) was administered by gastric gavage for 3 weeks and leuprolide depot formulation (1 mg/kg body weight) was injected once subcutaneously. Numbers within parentheses indicate number of treated animals. Differences from danazol-leuprolide combination; $p < 0.05$ in leuprolide alone, $p < 0.01$ in danazol alone.

With regard to formation of the fluid in the experimental endometriosis, all endometrial explants in 16 intact control rats accumulated fluid under the renal capsule. Conversely, complete resorption of the fluid accompanied by a marked atrophy of endometrial explant occurred in 13 of 14 ovariectomized rats (93%). In the remaining one the fluid accumulated. The fluid completely regressed in nine of 20 (45%) danazol-treated and in 14 of 24 (58%) leuprolide-treated animals. It was interesting to find that the fluid completely regressed in 16 of 17 (94%) animals by danazol-leuprolide combination. The therapeutic effect obtained in the combination was comparable to that obtained in castrated animals, and was significantly superior to that shown in animals treated with danazol ($p < 0.01$) or leuprolide ($p < 0.05$) alone. Serum concentrations of leuprolide and danazol in leuprolide- and danazol-treated animals were 260 ± 74 pg/ml and 31 ± 4 ng/ml, respectively. These concentrations in serum were similar to those in women who are given an effective dose of leuprolide or danazol.[11, 13]

To show the action mechanisms of these compounds on experimental endometriosis, serum concentrations of luteinizing hormone, follicle-stimulating hormone, and estradiol, and concentrations of pituitary GnRH receptors were determined under the treatment. Weights of uterus and ovaries were also measured (Table I). As expected, GnRH agonist leuprolide significantly ($p < 0.01$) decreased serum estradiol concentration as observed in castrated animals. However, serum estradiol was not decreased by danazol treatment. Serum estradiol value (38 ± 5 pg/ml) in the leuprolide-treated group was similar to the estradiol value (38 ± 4 pg/ml) in the danazol-leuprolide group (Table I). Uterine weight can be used as an index of estrogenic stimuli in female animals. We found that the changes in uterine weight under each treatment correlated well with those in serum estradiol concentrations. These results strongly suggest that the GnRH agonist leuprolide suppressed estradiol production and induced regression and atrophy of experimental endometriosis. The results also suggest that danazol possibly acted directly on the endometriotic tissues and induced atrophy. Therefore it is likely to consider that a greater response rate observed in danazol-leuprolide combination was obtained by the additive effects of danazol and leuprolide.

Despite the marked reduction in serum estradiol after leuprolide treatment, no decrease in radioimmunoassayable luteinizing hormone or follicle-stimulating hormone in serum was observed (Table I). However, leuprolide significantly reduced the concentration of pituitary GnRH receptors and the ovarian weight. These data suggest that the inhibitory effect of GnRH agonist leuprolide on estrogen production in female rats with endometriosis can be explained by a decrease in the secretion of pituitary bioactive gonadotropins but not of immunoreactive gonadotropins.

Comment

In a preliminary study endometriosis was induced in mature female rats by autotransplantation of a section of endometrium to the peritoneal wall as described by Jones.[14] However, we found some difficulties in evaluating the effects of treatment on the peritoneal endometriosis. As a result of peritoneal adhesion, the measurement of size of endometrial implant was difficult. Therefore in this study experimental endometriosis was induced under the capsule of kidney in mature female rats. The rate of induction of endometriosis in the kidney was >80%, and a precise evaluation of the effects of treatment could be achieved by

Table I. Concentrations of serum gonadotropins, serum estradiol, and pituitary GnRH receptors and weights of uterus and ovaries in control and treated groups

Treatment	Luteinizing hormone (ng/ml)	Follicle-stimulating hormone (ng/ml)	Estradiol (pg/ml)	GnRH receptors (fmol/mg protein)	Weight (mg)	
					Uterus	Ovaries
Control (n = 16)	0.9 ± 0.1	4.0 ± 0.4	73 ± 7	24 ± 5	212 ± 18	85 ± 4
Castrated (n = 14)	8.0 ± 1.2*	26.8 ± 1.7*	26 ± 7*	30 ± 5	63 ± 4*	—
Danazol (n = 20)	0.7 ± 0.1	3.9 ± 0.3	81 ± 19	18 ± 3	216 ± 14	83 ± 4
Leuprolide (n = 24)	0.7 ± 0.1	3.2 ± 0.2	38 ± 5*	3 ± 1*	75 ± 3*	50 ± 3*
Danazol-leuprolide (n = 17)	0.5 ± 0.1	3.7 ± 0.4	38 ± 4*	9 ± 2†	125 ± 8*	55 ± 5*

Numbers within parentheses indicate number of treated animals.
*Difference from control, $p < 0.01$.
†Difference from control, $p < 0.05$.

the present model. All endometrial explants in intact control were viable and were maintained essentially in the same size throughout the study. Removal of the ovaries resulted in a marked decrease in the size and atrophy of the explant in all animals except one. The findings show that this animal model is useful in evaluating the effects of therapy on endometriosis. Although use of both danazol and the GnRH agonist leuprolide, which are established compounds for treatment of endometriosis, were effective in decreasing the size and inducing atrophy and regression of the experimental endometriosis in approximately half the treated animals, combination therapy with danazol and leuprolide regressed the endometriosis in 94% of treated animals. The therapeutic effect with the combination was comparable to that induced by ovariectomy. These results suggest that combination therapy with danazol and GnRH agonist is a potential modality in the treatment of endometriosis, particularly for advanced stages of the disease.

A possible mechanism of action of the compound in endometriosis was also examined in this study. GnRH agonist leuprolide did not inhibit the secretion of immunoreactive gonadotropins but reduced ovarian weight and serum estradiol in female rats with endometriosis. Pituitary GnRH receptors in the animals were markedly down regulated after leuprolide treatment. These results suggest that leuprolide suppressed ovarian estrogen production through the secretion of a qualitatively different and biologically less active gonadotropin,[15] or through direct inhibition of ovarian steroid biosynthesis.[16] We recently found that suppression of estrogen production by GnRH agonist in patients with endometriosis is induced through the suppression of the secretion of bioactive luteinizing hormone, but not through direct inhibition of ovarian estrogen biosynthesis.[4] These results, together with those of a previous study in patients with endometriosis,[4] suggest that leuprolide suppressed the secretion of bioactive gonadotropin, which resulted in the suppression of ovarian estrogen production in animals with endometriosis.

In contrast to the GnRH agonist leuprolide, danazol did not decrease concentration of serum estradiol in animals with endometriosis. In nine female rats that responded to danazol and showed regression of endometriosis, decreases in serum estradiol concentrations were not observed (88 ± 19 vs 73 ± 7 pg/ml in intact control). The dose chosen for danazol in this study appears to be adequate and not suboptimal since the serum concentrations of danazol (31 ± 4 ng/ml) 24 hours after the last administration of the compound were in the same range of those in women receiving an effective dose of the compound in the treatment of endometriosis.[11, 13] It has been suggested that danazol produces anovulation and low levels of circulating estrogen and progesterone in women by acting at the hypothalamic, pituitary, and ovarian levels.[1] However, there are several reports[17, 18] showing that danazol does not suppress serum estrogen level in women of reproductive age. We[19] and others[7] found that short-term treatment with danazol induces atrophic changes in hyperplastic and normal endometrium without a decrease in the level of serum estradiol. One possible mechanism to support these findings is the marked increase in serum concentration of free testosterone during danazol treatment.[5] Testosterone has also been shown to inhibit proliferation of endometrial cells in culture.[20] A direct inhibitory effect of danazol on the growth of normal[8] and malignant endometrial cells[9] was also demonstrated. These results, combined with the previous results, suggest that danazol either directly or indirectly, through the increase of free testosterone, acted on endometriotic tissues in female rats and induced regression and atrophy. The combination therapy with danazol and GnRH agonist, which shows a different mode

of action, seems to have an advantage in the treatment of patients with advanced endometriosis. A clinical trial is under way in our department to clarify whether the combination therapy is a potential modality in the treatment of patients with advanced endometriosis.

REFERENCES

1. Barbieri RL, Ryan KJ. Danazol: endocrine pharmacology and therapeutic applications. Am J Obstet Gynecol 1981;141:453-63.
2. Lemay A, Maheux R, Faure N, Jean C, Fazekas ATA. Reversible hypogonadism induced by a luteinizing hormone-releasing hormone (LH-RH) agonist (buserelin) as a new therapeutic approach for endometriosis. Fertil Steril 1984;41:863-71.
3. Henzl MR, Corson SL, Moghissi K, Buttram VC, Berqvist C, Jacobson J. Administration of nasal nafarelin as compared with oral danazol for endometriosis—a multicenter double-blind comparative clinical trial. N Engl J Med 1988;318:485-9.
4. Ohtsuka S, Terakawa N, Shimizu I, et al. Studies on GnRH agonist suppression of estrogen production in patients with endometriosis. Endocrinol Jpn 1989;36:611-9.
5. Meldrum DR, Pardridge WM, Karow WG, Rivier J, Vale W, Judd HL. Hormonal effects of danazol and medical oophorectomy in endometriosis. Obstet Gynecol 1983; 62:480-5.
6. Kokko E, Jänne O, Kauppila A, Rönnberg L, Vihko R. Danazol has progestin-like actions on the human endometrium. Acta Endocrinol 1982;99:588-93.
7. Jeppsson S, Mellquist P, Rannevik G. Short term effects of danazol on endometrial histology. Acta Obstet Gynecol Scand1984;123(suppl):41-4.
8. Rose GL, White JO, Dowsett M, Jeffcoate SL, Mudge JE. The inhibitory effects of danazol, danazol metabolites, gestrinone, and testosterone on the growth human endometrial cells in vitro. Fertil Steril 1988;49:244-8.
9. Ikegami H, Terakawa N, Shimizu I, et al. Danazol binds to progesterone receptors and inhibits the growth of human endometrial cancer cells in vitro. Am J Obstet Gynecol 1986;155:857-61.
10. Okada H, Heya T, Ogawa Y, Shimamoto T. One-month release injectable microcapsules of a luteinizing hormone-releasing hormone agonist (leuprolide acetate) for treating experimental endometriosis in rats. J Pharmacol Exp Ther 1988;244:744-50.
11. Nygard GA, Lovett LJ, Erdmann GR, Khalil SKW. Analysis of danazol in serum using high-performance liquid chromatography. J Chromatogr 1987;415:438-44.
12. Shimizu I, Terakawa N, Aono T, Tanizawa O, Matsumoto K. Clomiphene citrate induces pituitary GnRH receptors in ovariectomized rats: its possible role in induction of ovulation. Acta Endocrinol 1986;11:179-84.
13. Davison C, Banks W, Fritz A. The absorption, distribution and metabolic fate of danazol in rats, monkeys and human volunteers. Arch Int Pharmacodyn Ther 1976;221:294-310.
14. Jones RC. The effect of a luteinizing hormone releasing hormone (LRH) agonist (Wy-40,972), levonorgestrel, danazol and ovariectomy on experimental endometriosis in the rat. Acta Endocrinol 1984;106:282-8.
15. Meldrum DR, Tsao Z, Monroe E, et al. Stimulation of LH fragments with reduced bioactivity following GnRH agonist administration in women. J Clin Endocrinol Metab 1984;58:755-7.
16. Tureck RW, Mastroianni L Jr, Blasco L, Strauss JF III. Inhibition of human granulosa cell progesterone secretion by a gonadotropin-releasing hormone agonist. J Clin Endocrinol Metab 1982;54:1078-80.
17. Hirscjowitz JS, Soler NG, Wortzman J. Sex steroid levels during treatment of endometriosis. Obstet Gynecol 1979; 54:448-50.
18. Tamaya T, Murakami T, Yamada T, Wada K, Fujimoto J, Okada H. Serum hormone and steroid receptor levels during luteal-phase and long-term treatment with danazol. Fertil Steril 1983;40:585-9.
19. Terakawa N, Inoue M, Shimizu I, et al. Preliminary report on the use of danazol in the treatment of endometrial adenomatous hyperplasia. Cancer 1988;62:2618-21.
20. Hiratsu T. In vitro cultivation of human endometrium and the influences of steroid hormones on a cell line derived from the endometrium. Kobe J Med Sci 1968;14:29-48.

Uterine artery estrogen receptors in the nonpregnant and pregnant guinea pig

Joseph R. Leiberman, MD,[a] Cor C. J. van Vroonhoven, Tech.,[b] Ilse Beckmann, PhD,[c] Theo H. van der Kwast, MD,[b] and Henk C. S. Wallenburg, MD, PhD[c]

Beer Sheva, Israel, and Rotterdam, The Netherlands

Uterine artery, heart, and aorta or carotid specimens of nonpregnant, midpregnant, and term pregnant guinea pigs were examined for estrogen receptors by immnocytochemical methods. Estrogen receptors were found in the nuclei of cells in the endothelial, muscle, and adventitia layers of the uterine artery wall. The concentration of estrogen receptors was slightly higher in nonpregnant and term pregnant animals than in midpregnancy. No estrogen receptors were found in the heart, aorta, or carotid specimens of all animals. These results confirm the uterine artery as a target organ of estrogen action that would eventually lead to arterial functional adaptation in different biologic periods. (Am J Obstet Gynecol 1990;163: 1685-8.)

Key words: Uterine artery, estrogen receptor, guinea pig, estrogen action, immunocytochemical methods

It has been shown in various experiments that estrogens induce dilatation of the uterine arteries in women,[1] in the rhesus monkey,[2] the guinea pig,[3] and the ewe.[4] In the guinea pig a single injection of a medium long-acting estrogen provokes a typical widening of the mesometrial arteries[5] and stimulates deoxyribonucleic acid synthesis.[6] The effects of estrogens on the reproductive tissues are mediated by intracellular receptors.[7] Steroid-binding assays[8, 9] have been used to quantify estrogen receptor concentrations in uterine arteries, but the necessary homogenization of tissues prevented identification of cells containing estrogen receptors. The recent development of immunocytochemical methods that use monoclonal antibodies allows the intracellular detection[10, 11] and tissue localization of estrogen receptors.

The purpose of this study was to assess the presence and concentration of estrogen receptors in the uterine arterial wall of nonpregnant and pregnant guinea pigs.

Material and methods

Tissues were collected from four nonpregnant and seven pregnant albino (Hartley) guinea pigs bred in our laboratory. The guidelines for the care and use of the animals approved by the Erasmus University were followed. The animals were kept in individual cages in a controlled environment (19° C, 50% humidity) with a light/dark cycle of 13 hours/11 hours. Animals were fed commercial guinea pig pellets, hay, and water ad libitum. Vitamin C was added to the drinking water twice a week. To remove various tissues for investigation, the animals were anesthetized with ketamine hydrochloride (30 mg/kg) and xylazine (3 mg/kg) administered intramuscularly. Lidocaine 2% was added subcutaneously at the incision site. At the time of operation three of the pregnant animals were at midpregnancy (between 25 and 35 days after mating) and four were at term (between 55 and 65 days pregnant). Segments of 10 mm length were taken from the left and right uterine arteries at the proximal and distal ends. The number of fetuses in each horn was counted. Endometrium and myometrium were sampled as reference tissues. Finally, tissue samples were taken from the general vascular system, including the heart and aorta or carotid. The animals were killed by exsanguination, and blood was collected, centrifuged, and the plasma was stored at −20° C for hormone level determination. Estradiol and progesterone levels were measured by radioimmunoassay methods described previously.[12, 13]

Specimens were partially fixed in buffered 4% formaldehyde, dehydrated, and embedded in paraffin for conventional histologic study. The remaining parts were immediately frozen in chilled isopentane and stored in liquid nitrogen until use. Cryostat sections with a thickness of 6 μm were cut and fixed in a 4% formaldehyde–phosphate-buffered saline solution, pH

From the Division of Obstetrics and Gynecology, Soroka University Hospital, Beer Sheva,[a] and the Department of Pathology[b] and the Department of Obstetrics and Gynecology,[c] Erasmus University, Rotterdam.

Received for publication December 8, 1989, revised June 13, 1990; accepted July 16, 1990.

Reprint requests: Joseph R. Leiberman, MD, Division of Obstetrics and Gynecology, Soroka University Hospital, POB 151, Beer Sheva, Israel.

6/1/23817

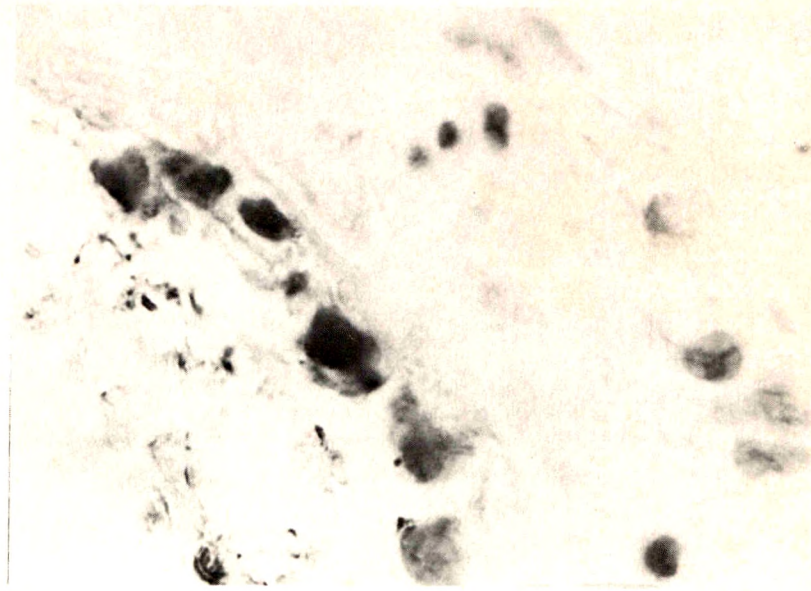


Fig. 1. Light micrograph of guinea pig uterine artery wall shows estrogen receptors with nuclear localization. (Original magnification ×1000.)

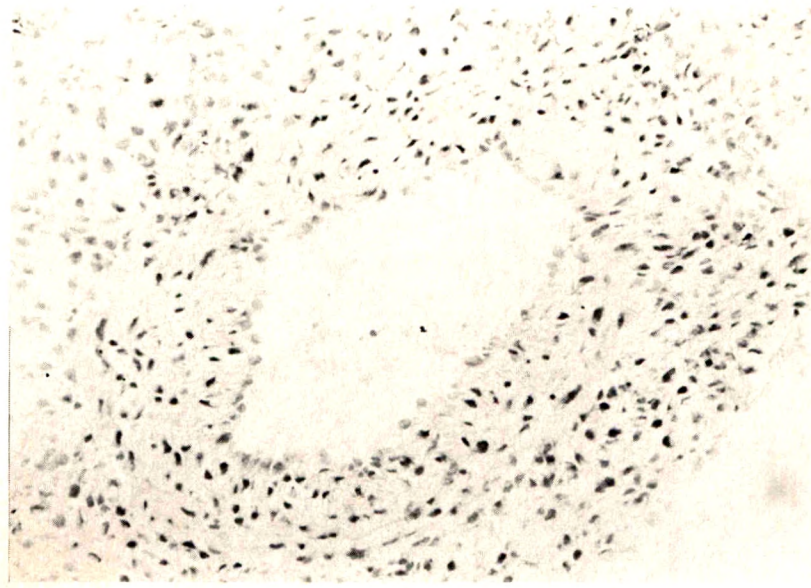


Fig. 2. Light micrograph shows distribution of estrogen receptors in endothelial, muscle cell, and adventitia layers of guinea pig uterine artery wall. (Original magnification ×250.)

7.4, for 10 minutes at room temperature. After fixation the slides were incubated in chilled methanol and acetone, both at −20° C for 5 minutes and washed in phosphate-buffered saline solution (pH 7.4) for 10 to 15 minutes. The tissues were incubated with a monoclonal antiestrogen receptor antibody by means of the Abbott ER-ICA kit (Abbott, North Chicago, Ill.). The Abbott kit protocol was followed. The kit contains nonimmune goat serum, primary rat-monoclonal antiestrogen receptor antibody, linking goat-antirat antibody and the rat peroxidase-antiperoxidase complex. The immunocomplex was visualized with 3,3′-diaminobenzidine tetrahydrochloride. Two of the authors independently evaluated the staining semiquantitatively by scoring the percentage of stained cells in the tissue.

Results

Details of animal weights and plasma hormone levels are shown in Table I. The horns of the pregnant ani-

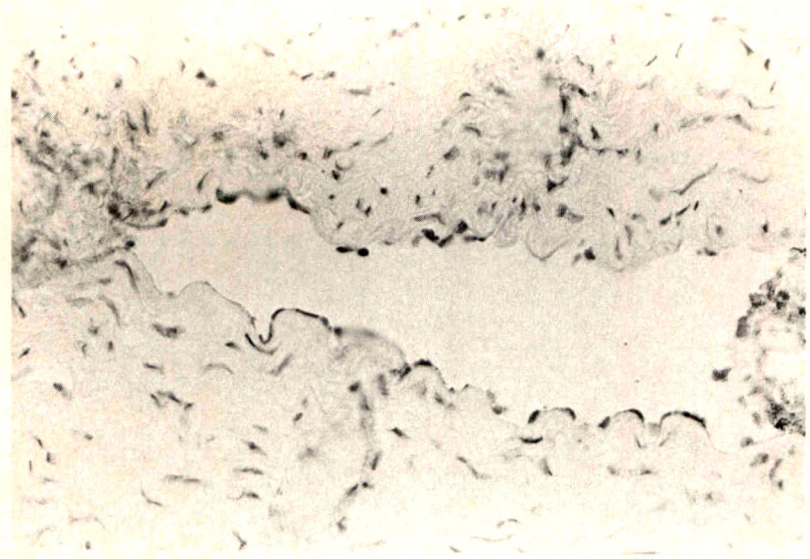


Fig. 3. Light micrograph of guinea pig carotid artery shows no estrogen receptors. (Original magnification ×250.)

mals contained two to five fetuses. Immunocytochemical staining revealed estrogen receptors in the nucleus of the cells in endothelium, muscle, and adventitia layers of the uterine arterial wall in all three groups of animals. The percentage of stained cells was slightly higher in the nonpregnant (stained cells: 35.0% ± 16.2%, mean ± SD) and term pregnant (33.6% ± 19.8%, mean ± SD) animals than in the animals in midpregnancy (19.0% ± 17.4%, mean ± SD). A trend to higher percentages of stained cells was found in the adventitia in all three groups of animals. There were no animals without estrogen receptors in the uterine artery wall. Similar concentrations of estrogen receptors were found in the endothelial and muscle cell layers in most uterine artery segments. Proximal and distal segments of the uterine artery showed no difference in the relative number of estrogen receptors. Left and right uterine arteries had similar percentages of stained cells, and the number of fetuses in each horn had no influence on staining. The amount of stained cells of all animals was 49.9% ± 13.5% (mean ± SD) in the endometrium and 37.7% ± 4.4% (mean ± SD) in the myometrium. Heart, aorta, and carotid showed no estrogen receptors in any of the animals in the three groups.

Table I. Weight and hormone plasma levels in nonpregnant and pregnant guinea pigs

	Weight (gm)	*Estradiol (pmol/L)*	*Progesterone (nmol/L)*
Nonpregnant			
Median	787	27	11
Range	694-956	11-77	11-28
Midpregnant			
Median	777	8	340
Range	709-855	8-26	5-707
Termpregnant			
Median	1244	189	717
Range	1074-1394	143-355	159-1520

Comment

By means of immunocytochemical staining we have demonstrated the presence of estrogen receptors in the uterine artery wall of the nonpregnant and the pregnant guinea pig. The insoluble chromogen produced by the staining method was easily visualized within the nuclei of the cells. We did not assess the staining intensity because small differences in thickness of the specimen may induce optically false images of the chromogen concentration. The estrogen receptors were found in the intima, media, and adventitia of the guinea pig uterine artery. Lessey et al.[14] examined uterine tissue samples from women undergoing hysterectomy and found that vascular tissue within the sections did not bind antireceptor antibody at any stage of the cycle. In contrast, Perrot-Aplanat et al.[15] found estrogen and progesterone receptors in muscle cells of the rabbit uterus arterial bed. These authors also observed estrogen and progesterone receptors in smooth muscle cells in human endometrial spiral arteries, with strong staining at the end of the luteal phase.

We found slightly higher percentages of stained cells in uterine artery specimens from nonpregnant and term pregnant animals than in those obtained in midpregnancy. The scores for endometrial and myometrial staining were similar in all groups. This suggests a

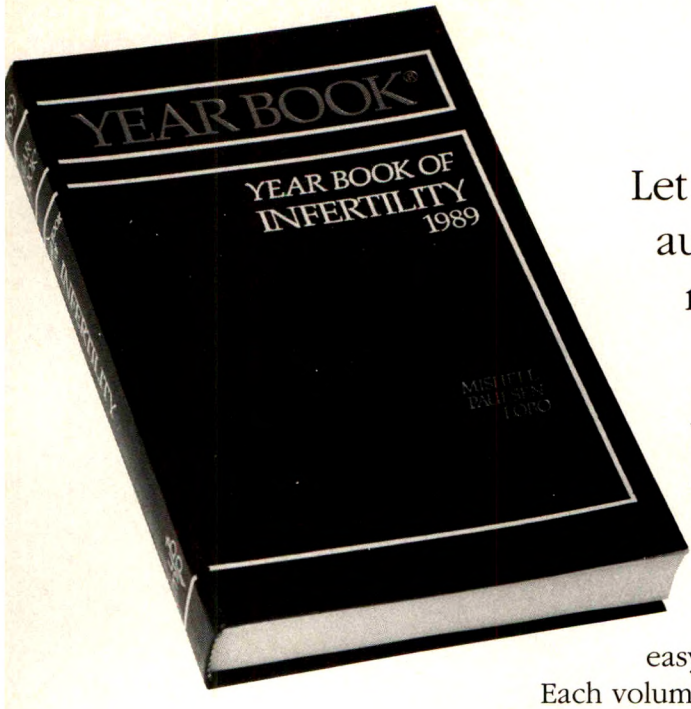
change in estrogen sensitivity of the uterine artery in different periods of gestation, which may be related to the high progesterone/estradiol ratio we found in midpregnancy (see Table I). Because of the small number of animals, the differences were not statistically assessed.

We were not able to demonstrate estrogen receptors in the heart, aorta, and carotid. This is in agreement with Perrot-Aplanat et al.,[15] who did not observe estrogen or progesterone receptors in the rabbit myocardium, aorta, or intestinal, renal, hepatic, femoral, and pulmonary arteries. However, estrogen binding has been described by autoradiography in the muscle cells of the media of the renal artery in the rat.[16] Estradiol binding was found in rat aortic smooth muscle cells in culture.[17] Horwitz and Horwitz[18] observed estrogen binding sites in the media, adventitia, or both of the dog aorta. Estrogen binding sites were observed also in endothelial cell cultures.[19] The presence of estrogen receptors has been reported in human aorta.[20] Thus the absence of estrogen receptors in the heart, aorta, and carotid of the guinea pig may be explained by species variation or could perhaps be related to the sensitivity of the method.

We thank Frank H. de Jong, PhD, for the estimations of steroids.

REFERENCES

1. Borell U, Fernstrom I, Westman A. Hormonal influence in the uterine arteries: an arteriographic study in the human. Acta Obstet Gynecol Scand 1953;32:271-84.
2. Markee JE. Menstruation in intraocular endometrial transplants in the Rhesus monkey. Contrib Embryol Carnegie Inst 1940;28:219-308.
3. Bell C. Oestrogen-induced sensitization of the uterine artery of the guinea pig to acetylcholine. Br J Pharmacol 1973;49:595-601.
4. Resnik R, Killam AP, Battaglia FC, Makowski EL, Meschia G. The stimulation of uterine blood flow by various estrogens. Endocrinology 1974;94:1192-6.
5. Moll W, Gotz R. Pressure-diameter curves of mesometrial arteries of guinea pigs demonstrate a non-muscular oestrogen-inducible mechanism of lumen regulation. Pflügers Arch 1985;404:332-6.
6. Makinoda S, Moll W. DNA synthesis in mesometrial arteries in guinea pigs during oestrus cycle, pregnancy and under treatment with oestradiol benzoate. Placenta 1986; 7:189-98.
7. Eriksson H, Gustafsson JA. Steroid hormone receptors: structure and function. Amsterdam: Elsevier Biomedical, 1983:389.
8. Lantta M, Karkkainen J, Lehtovirta P. Progesterone and estradiol receptors in the cytosol of the human uterine artery. Am J Obstet Gynecol 1983;147:627-33.
9. Batra S, Iosif S. Nuclear estrogen receptors in human uterine arteries. Gynecol Obstet Invest 1987;24:250-5.
10. King WJ, Greene GL. Monoclonal antibodies localize oestrogen receptors in the nuclei of target cells. Nature 1984;307:745-7.
11. Welshons WV, Lieberman ME, Gorsky J. Nuclear localization of unoccupied oestrogen receptors. Nature 1984; 307:747-9.
12. Van Hemert AM, Birkenhager JC, De Jong FH, Vanderbroucke JP, Valkenburg HA. Sex hormone binding globulin in postmenopausal women: a predictor of osteoporosis superior to endogenous oestrogens. Clin Endocrinol [In press].
13. De Jong FH, Baird DT, Van de Molen HJ. Ovarian secretion rates of oestrogens, androgens and progesterone in normal women and in women with persistent ovarian follicles. Acta Endocrinol 1974;77:575-87.
14. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty KS Jr. Immunocytochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab 1988;67:334-40.
15. Perrot-Aplanat M, Groyer-Picard MT, Garcia E, Lorenzo F, Milgrom E. Immunocytochemical demonstration of estrogen and progesterone receptors in muscle cells of uterine arteries in rabbits and humans. Endocrinology 1988; 123:1511-9.
16. Stumpf WE, Sar M. Autoradiographic localization of estrogen, androgen, progestin and glucocorticosteroid in "target tissues" and "nontarget tissues". In: Pasqualini J, ed. Receptors and mechanisms of action of steroid hormones. New York: Marcel Dekker, 1976:41.
17. Nakao J, Chang WC, Murota SI, Orimo H. Estradiol-binding sites in rat aortic smooth muscle cells in culture. Atherosclerosis 1981;38:75-80.
18. Horwitz KB, Horwitz LD. Canine vascular tissues are targets for androgens, estrogens, progestins and glucocorticoids. J Clin Invest 1982;69:750-8.
19. Colburn P, Buonassisi V. Estrogen-binding sites in endothelial cell cultures. Science 1978;201:817-9.
20. Campisi D, Bivona A, Paterna S, Valenza M, Albiero R. Oestrogen binding sites in fresh human aortic tissue. Int J Tissue React 1987;9:393-8.



Let our eminent medical authorities guide you through major new clinical advances

Introducing the

Year Book of Infertility®

This time-saving guide book makes it easy for you to keep current in your field. Each volume represents the year's most significant developments as reported in more than 800 journals and periodicals published worldwide. These selections are condensed to roughly a page in length, so you get all essential facts at a glance.

More importantly, the distinguished physician editors provide thoughtful, probing commentaries that explore the significance of each article while evaluating its practical implications for day-to-day practice.

Save hours of reading and research time and get expert guidance with this invaluable reference!

These books are published annually. For the convenience of its customers, Year Book enters each purchaser as a subscriber to future volumes and sends annual announcements of each volume approximately two months before publication. The new volume will be shipped upon publication unless you complete and return the cancellation notice attached to the announcement and it is received by Year Book within the time indicated (approximately 20 days after your receipt of the announcement). You may cancel your subscription at any time. The new volume may be examined on approval for 30 days and returned for full credit. Year Book will remove your name as a subscriber. Return postage is guaranteed by Year Book to the Postal Service.

To Subscribe:

Simply fill out this coupon and send it to us at the address shown.

Or,

for faster service, call our TOLL-FREE number:

1-800-426-4545

Prepaid orders are shipped postage free; add $3.50 per order to cover handling. Other orders will be billed a shipping and handling charge. IL, MA, TN residents will be billed appropriate sales tax. Prices are quoted in U.S. dollars. Canadian orders billed in U.S. funds. All prices subject to change without notice.

[✗] ***YES!***

I know it's vital that I stay informed. Please rush me the most recent issue of the *Year Book of Infertility* (IN-89) for $54.95.

❒ Payment Enclosed ❒ Please bill me ❒ Charge my Amex/Visa/MasterCard (circle one)

Signature

Account Number/Expiration Date

Name

Address

City/State/Zip

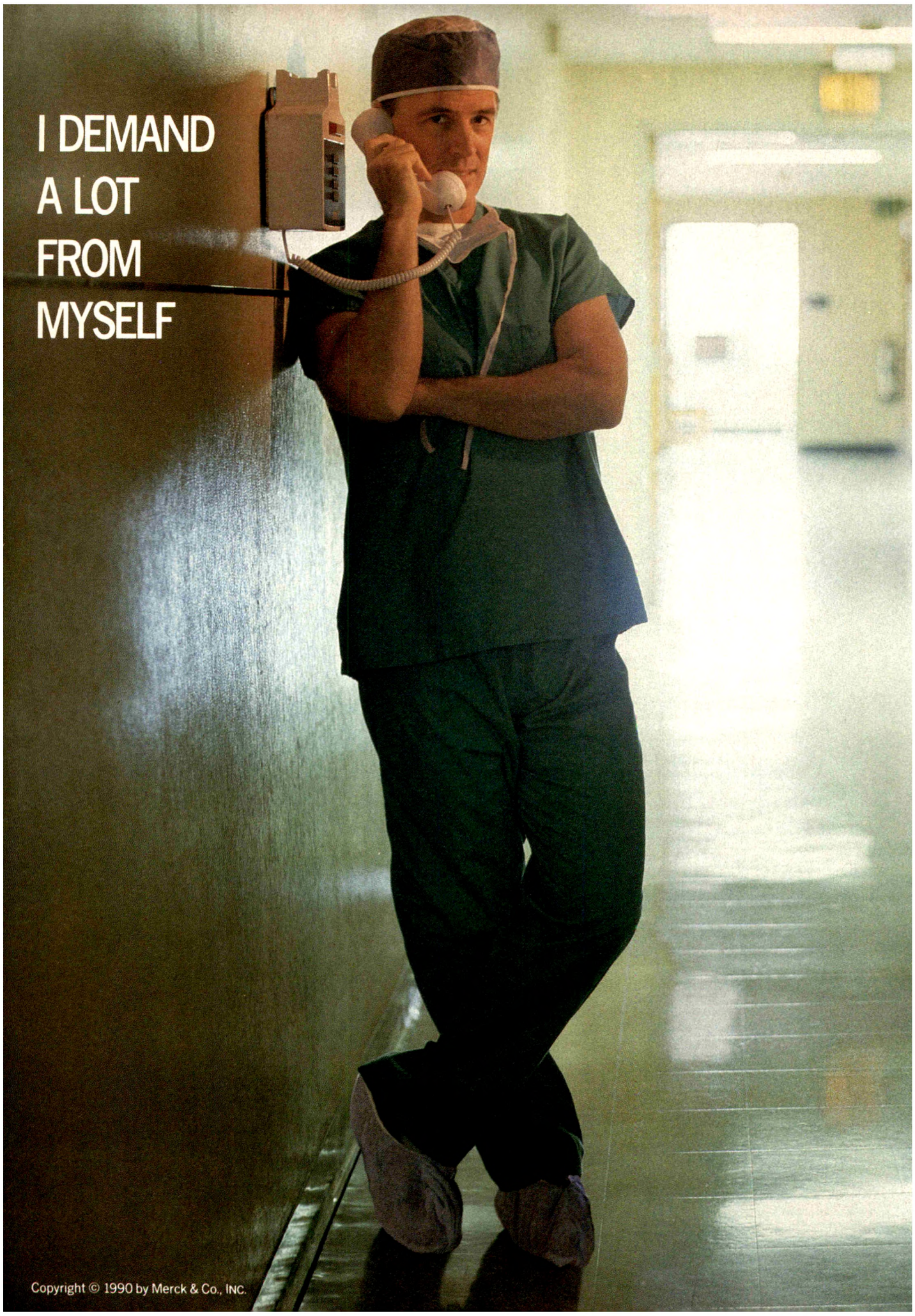
Telephone XX7

Mail to:

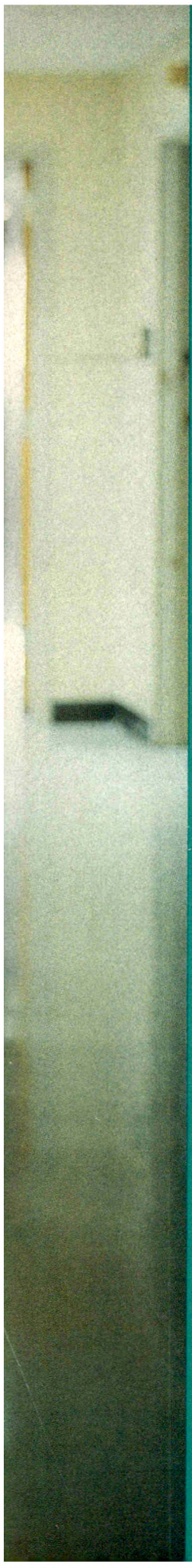
Mosby-Year Book Inc.

Order Processing

11830 Westline Industrial Dr. St. Louis, MO 63146



Copyright © 1990 by Merck & Co., INC.



I DEMAND A LOT FROM AN ANTIBIOTIC

MEFOXIN

...with well-documented clinical experience*

...broad coverage of important aerobes and anaerobes, including the *B. fragilis* group

...used in over 14 million patients.

BALANCED COVERAGE† WITH

MEFOXIN® IV IM

(CEFOXITIN SODIUM | MSD)

†*in vitro* activity against indicated aerobes and anaerobes

MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

MEFOXIN is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Streptococcus faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to MEFOXIN.

*in infections caused by indicated organisms at indicated sites

Please see following page for a Brief Summary of Prescribing Information.

BALANCED COVERAGE† WITH

MEFOXIN® IV IM

(CEFOXITIN SODIUM | MSD)

†*in vitro* activity against indicated aerobes and anaerobes

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae,* other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli, Klebsiella* species, *Hemophilus influenzae,* and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli, Klebsiella* species, *Proteus mirabilis,* indole-positive *Proteus* (which include the organisms now called *Morganella morganii* and *Proteus vulgaris*), and *Providencia* species (including *Providencia rettgeri*). Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli, Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,* and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli, N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,* *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae, Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli, Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.*

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis,* streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli, P. mirabilis, Klebsiella* species, *Bacteroides* species including the *B. fragilis group,** *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae.* Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Prevention—Prophylactic use in surgical procedures (e.g., hysterectomy, gastrointestinal surgery, cesarean section, transurethral prostatectomy) classified as contaminated or potentially contaminated or in patients in whom subsequent infection at the operative site would present a serious risk, e.g., prosthetic arthroplasty. MEFOXIN usually should be given ½ to 1 hour before surgery and should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions but, in the majority of surgical procedures, does not reduce the incidence of subsequent infection. However, in patients undergoing prosthetic arthroplasty, it is recommended that MEFOXIN be continued for 72 hours after the surgical procedure. If there are signs of infection, specimens for culture should be obtained for identification of the causative organism so appropriate treatment may be instituted.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxycorticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST** reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase; jaundice. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials, infusion bottles, and ADD-Vantage®† vials containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus

**Registered trademark of Ames Company, Division of Miles Laboratories, Inc.
†Registered trademark of Abbott Laboratories, Inc.

For more detailed information, consult your MSD Representative or see Prescribing Information. Merck Sharp & Dohme, Division of Merck & Co., INC., West Point, PA 19486 JOMF 74(126)

MSD
MERCK SHARP & DOHME

CONSENSUS REPORT

National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy

The following paper represents a consensus by the Working Group on High Blood Pressure in Pregnancy, convened in 1988, by the National High Blood Pressure Education Program, National Heart, Lung, and Blood Institute, Bethesda, Maryland.

The publication of this report is supported by an educational grant from Merck Sharp & Dohme.

Foreword

This statement on high blood pressure in pregnancy represents a consensus between obstetricians and internists on a controversial topic in which no consensus existed previously.

The National High Blood Pressure Education Program of the National Heart, Lung, and Blood Institute, working with the American College of Obstetricians and Gynecologists, appointed a working group of physicians and scientists with expertise and experience in either hypertension or pregnancy or both. The group met five times between September 1988 and May 1989, and between the regular meetings members corresponded with each other by telephone, fax, and U.S. mail, exchanging ideas and drafts of chapters. The resulting document has been reviewed and approved by both the Coordinating Committee of the National High Blood Pressure Educational Program and the Committee on Obstetrics: Maternal and Fetal Medicine of the American College of Obstetricians and Gynecologists.

6/0/24960

The members of the Working Group were conscientious in carrying out their assignments and faithful in attending the meetings. Special citation goes to Leon Chesley, PhD, who devoted a distinguished career to this subject and whose perspective, knowledge, and good humor added immeasurably to our deliberations.

Claude Lenfant, MD
Chairman, National High Blood Pressure Education Program Coordinating Committee
Director, National Heart, Lung, and Blood Institute

Ray W. Gifford, Jr., MD
Chairman of the Working Group
American Medical Association Representative to the National High Blood Pressure Education Program Coordinating Committee

Frederick P. Zuspan, MD
Member of the Working Group
Editor, AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

Members of the Working Group on High Blood Pressure in Pregnancy

Ray W. Gifford, Jr., MD
Vice Chairman, Division of Medicine
Senior Physician, Department of Hypertension and Nephrology
Cleveland Clinic Foundation
Cleveland, Ohio

Phyllis August, MD
Assistant Professor of Medicine and Obstetrics and Gynecology
Cornell University Medical College
New York Hospital
New York, New York

Leon C. Chesley, PhD
State University of New York
Brooklyn, New York

Gary Cunningham, MD
Professor and Chairman
Department of Obstetrics and Gynecology
University of Texas Southwestern Medical Center
Dallas, Texas

Thomas F. Ferris, MD
Nesbitt Professor and Chairman
Department of Medicine
University of Minnesota Medical School
Minneapolis, Minnesota

Marshall D. Lindheimer, MD
Professor of Medicine and Obstetrics and Gynecology
University of Chicago
Pritzker School of Medicine
Chicago, Illinois

C. W. G. Redman, FRCP
Reader in Obstetric Medicine and Gynecology
Department of Obstetrics and Gynecology
John Radcliff Hospital
Headington, Oxford, United Kingdom

James M. Roberts, MD
Professor of Obstetrics, Gynecology, and Reproductive Sciences
Senior Staff, Cardiovascular Research Institute
University of California, San Francisco
San Francisco, California

Frederick P. Zuspan, MD
Professor, Richard L. Meiling Chair
Department of Obstetrics and Gynecology
Ohio State University
Columbus, Ohio

Ex officio members

Michael J. Horan, MD, ScM
Associate Director for Cardiology
Division of Heart and Vascular Diseases
National Heart, Lung, and Blood Institute
Bethesda, Maryland

Donald McNellis, MD
Special Assistant for Obstetrics
Pregnancy and Perinatology Branch
National Institute of Child Health and Human Development
Bethesda, Maryland

Edward J. Roccella, PhD, MPH
Coordinator, National High Blood Pressure Education Program
National Heart, Lung, and Blood Institute
Bethesda, Maryland

Staff

Mary McDonald, RN, MS
University Research Corporation
Bethesda, Maryland

The members of the Working Group on High Blood Pressure and Pregnancy also gratefully acknowledge the contributions of Kathleen Auerbach, PhD, IBCLC, Ray Borzanian, Jane Rusche, RN, BS, and William B. White, MD.

Member organizations of the National High Blood Pressure Education Program Coordinating Committee

American Academy of Family Physicians
American Academy of Ophthalmology
American Academy of Physician Assistants
American Association of Occupational Health Nurses
American College of Cardiology
American College of Chest Physicians
American College of Physicians
American College of Preventive Medicine
American Dental Association
American Diabetes Association, Inc.
American Dietetic Association
American Heart Association
American Hospital Association
American Medical Association
American Nurses' Association, Inc.
American Occupational Medical Association
American Optometric Association
American Osteopathic Association
American Pharmaceutical Association
American Podiatric Medical Association
American Public Health Association
American Red Cross
American Society of Hospital Pharmacists
American Society of Hypertension
Association of Life Insurance Medical Directors of America
Citizens for the Treatment of High Blood Pressure, Inc.
National Black Nurses' Association, Inc.
National Heart, Lung, and Blood Institute
National Heart, Lung, and Blood Institute Ad Hoc Committee on Minority Populations
National Hypertension Association, Inc.
National Kidney Foundation
National Medical Association
National Optometric Association
Society for Nutrition Education

National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy

This consensus report focuses the presentation, pathophysiology, and management of the hypertensive disorders of pregnancy expanding on recommendations first presented in 1988 by the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Practicing physicians should determine whether a patient's hypertension during pregnancy falls into the classification of (1) chronic hypertension, (2) preeclampsia, (3) preeclampsia superimposed on chronic hypertension, or (4) transient hypertension. The distinction, for management considerations, is made between hypertension that is present before pregnancy (chronic and preexisting) and that occurring as part of the pregnancy-specific condition preeclampsia. When maternal blood pressure reaches diastolic levels of 100 mm Hg or greater, treatment should be instituted to avoid hypertensive vascular damage. The report includes a discussion of antihypertensive therapy specific to the chronic or acute hypertension occurring concomitantly with pregnancy. The roles of calcium supplementation and low-dose aspirin to prevent preeclampsia and chronic and transient hypertension are under investigation. (Am J Obstet Gynecol 1990;163:1689-1712.)

Key words: Pregnancy, hypertension, preeclampsia

Executive summary

High blood pressure complicates almost 10% of all pregnancies, and the incidence is higher if the women are nulliparous or carrying multiple fetuses. Causes of hypertension in pregnancy are multiple, and many current and previously used classification systems are elaborate and confusing, in part because of difficulty in making an etiologic diagnosis by clinical criteria alone. The consensus group recommends the schema proposed by the American College of Obstetrics and Gynecologists (ACOG) in 1972, which has been in wide use for some time, is practical and concise, and considers hypertension associated with gestation in only four categories: (1) chronic hypertension (of whatever cause but mainly essential), (2) preeclampsia, (3) preeclampsia superimposed on chronic hypertension, and (4) transient hypertension.

Preeclampsia (pure or superimposed) represents the greatest danger for the fetus and is associated with life-threatening maternal syndromes, whereas transient hypertension is a fairly benign disorder characterized by mild to moderate elevations of blood pressure late in pregnancy that return to normal post partum. Essential hypertension, also, is usually well tolerated if elevations remain (with or without therapy) below diastolic levels* of 100 mm Hg (Korotkoff phase V), but complications such as midtrimester loss, growth retardation, and abruptio placentae may occur more frequently. Because the preeclamptic syndromes and essential hypertension constitute more than three quarters of the hypertensive disorders in pregnancy, this document focuses on the presentation, pathophysiology, and management of these diseases.

Preeclampsia, a disease peculiar to pregnancy, mainly in nulliparous women, occurs primarily after gestational week 20, most frequently near term. Signs helpful in its diagnosis are presence of proteinuria, edema (especially if of recent and rapid onset), and any of the following: hemoconcentration, hypoalbuminemia, hepatic function or coagulation abnormalities, and increased urate levels. The predictive value of raised serum iron and low antithrombin III levels and hypocalciuria is under investigation.

A major pathophysiologic feature of this disease is a marked increase in peripheral resistance. This vasospasm is, in part, the result of exaggerated vascular responsiveness to circulating angiotensin II and catecholamines (and possibly an imbalance between thromboxane and prostacyclin production). Before intervention, cardiac output is often decreased, pulmonary capillary wedge pressure is normal or low, and intravascular volume is below that of normal pregnant women. Renal hemodynamics also decrease in part because of a characteristic morphologic lesion in the glomerulus, and there may be increased vascular permeability leading to albumin loss from the intravascular space. Uteroplacental perfusion is often compromised, which may lead to fetal growth retardation.

Preeclampsia can lead to two life-threatening complications. The first is a rapidly developing syndrome characterized by microangiopathic hemolytic anemia and marked signs of liver dysfunction, as well as coagulation changes. This variant, termed HELLP (hemolysis, elevated liver enzymes, low platelet count), constitutes an emergency requiring prompt termina-

*In this document diastolic blood pressure is defined as the onset of Korotkoff phase V (disappearance of sound).

tion of pregnancy. The second complication is progression of preeclampsia to a convulsive phase termed eclampsia, at one time the major cause of cerebral bleeding and maternal death in this disorder. Pending or frank eclampsia also requires immediate termination of gestation.

Therapy for preeclampsia when gestation is advanced is delivery. Severe intrapartum hypertension is controlled with intravenous hydralazine, which is successful in most instances. Favorable results have been recorded with parenteral diazoxide, labetalol, and clonidine as well as oral nifedipine. Nitroprusside should be avoided unless maternal jeopardy is extreme. Magnesium sulfate remains the drug of choice to prevent or treat eclamptic convulsions.

Women with essential hypertension often have reductions in blood pressure during the first two trimesters; failure for this to occur is an unfavorable prognostic sign. When pharmacologic intervention is required, α-methyldopa, because of its long history of safe use and a trial including a 7-year follow-up of the neonate, remains the drug of choice to treat chronic hypertension in pregnancy. Data relevant to β-blocking agents and combined β- and α-adrenergic receptor antagonists demonstrate their usefulness with little or no evidence of fetal jeopardy. Thiazide diuretics, which have been used safely in normotensive gravid women, may be used in hypertensive pregnant women, especially those who are sensitive to salt. Preliminary information concerning calcium channel antagonists is also encouraging, but angiotensin-converting enzyme inhibitors are contraindicated in pregnant women.

Finally, like all pregnant women, those with hypertension should refrain from alcohol and tobacco. The roles of calcium supplementation and low-dose aspirin (which decreases thromboxane synthesis while sparing prostacyclin production) to prevent preeclampsia and chronic and transient hypertension are under investigation.

Introduction

The hypertensive disorders during pregnancy are important causes of maternal death throughout the world, and most of these deaths are attributed to eclampsia. The hypertensive disorders also contribute extensively to stillbirths and neonatal morbidity and death. Hypertensive expectant mothers (or gravidas) are predisposed to the development of potentially lethal complications, notably abruptio placentae, disseminated intravascular coagulation, cerebral hemorrhage, hepatic failure, and acute renal failure.

The prevalence of chronic hypertension in pregnancy (that is, in those hypertensive women who become pregnant) is not known. It differs widely in different geographic areas but is probably present in 1% to 5% of all pregnancies.[1] The number of women who become hypertensive during pregnancy (preeclamptic) is also unclear, but one estimate from an indigent population is calculated to be about 13%,[2] and incidences ranging from 10% to 20% have been noted in nulliparous women.[3-6]

The purpose of this report is to provide guidance to the practicing physician in (1) managing hypertensive patients who become pregnant (hypertension that is present and observable before pregnancy or diagnosed before the twentieth week of gestation) and (2) managing pregnant patients who become hypertensive (the pregnancy-specific condition termed preeclampsia). This report expands on the recommendations of The 1988 Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure.[7]

Classification of hypertensive disorders of pregnancy

The most important consideration in the classification of increased blood pressure during pregnancy is the differentiation of hypertension that antedates pregnancy from a pregnancy-specific condition, which is characterized by poor perfusion of many organs and usually has increased blood pressure as one of its features. In the former condition, elevated blood pressure is the cardinal pathophysiologic feature, whereas in the latter increased blood pressure is important primarily as a sign of the underlying disorder. As might be expected, the impact of these two conditions on mother and fetus is quite different, as is their management. Unfortunately, attempts to differentiate these two classes of patients have led to confusion in terminology worldwide. The system proposed by the ACOG in 1972,[8] although imperfect, has been in wide use for some time and has the advantage of clarity and simplicity.* According to this terminology, women with increased blood pressure are divided into the following groups:

- Chronic hypertension
- Preeclampsia-eclampsia
- Preeclampsia superimposed on chronic hypertension
- Transient hypertension

Chronic hypertension. Chronic hypertension is defined as hypertension that is present and observable before pregnancy or that is diagnosed before the twentieth week of gestation. Hypertension is defined as a blood pressure equal to or greater than 140/90 mm

*More recent classifications are available from the ACOG,[9] World Health Organization (WHO),[10] and the International Society for the Study of Hypertension.[11] This working group elected to use this earlier classification scheme for purposes of clarity.

Hg. Hypertension that is diagnosed for the first time during pregnancy and persists beyond the forty-second day post partum is also classified as chronic hypertension.

Preeclampsia-eclampsia. The pregnancy-specific condition is termed preeclampsia in the ACOG classification[8] and usually occurs after 20 weeks' gestation (or earlier with trophoblastic diseases such as hydatidiform mole or hydrops). It is determined by increased blood pressure accompanied by proteinuria, edema, or both. Either of the following criteria suffice for the diagnosis of hypertension in this situation: (1) systolic blood pressure increases of 30 mm Hg or greater or (2) diastolic blood pressure increases of 15 mm Hg or greater from early values (average of values before 20 weeks' gestation). If prior blood pressure is not known, readings of 140/90 mm Hg or greater after 20 weeks' gestation are considered sufficiently elevated to satisfy the blood pressure criteria of preeclampsia. Note, however, that many young pregnant women will show the blood pressure increase required for the diagnosis of preeclampsia without increasing their pressure to 140/90 mm Hg.

In preeclampsia and eclampsia the blood pressure often is widely variable from moment to moment, and two observers measuring the blood pressure successively may obtain very different readings

Proteinuria is defined as the excretion of 0.3 gm or greater in a 24-hour specimen. This will usually correlate with 30 mg/dl ("1+ dipstick") or greater in a random urine determination. Proteinuria usually is a late sign in the course of preeclampsia; although it is nonspecific, its appearance greatly bolsters the diagnosis of preeclampsia. Edema is diagnosed as clinically evident swelling, but fluid retention may also be manifested as a rapid increase of weight without evident swelling.

Preeclampsia always presents potential danger to mother and infant. However, certain signs are particularly ominous:

- Blood pressure of 160 mm Hg or more systolic or 110 mm Hg or more diastolic
- Proteinuria of 2.0 gm or more in 24 hours (2+ or 3+ on qualitative examination); *the proteinuria should occur for the first time in pregnancy and regress after delivery*
- Increased serum creatinine values (>1.2 mg/dl unless known to be elevated previously)
- Platelet count less than 100,000 μl or evidence of microangiopathic hemolytic anemia (with increased lactic acid dehydrogenase)
- Elevated hepatic enzymes (alanine aminotransferase or aspartate aminotransferase)
- Headache or other cerebral or visual disturbances
- Epigastric pain
- Retinal hemorrhage, exudates, or papilledema; *these are extremely rare and it is most unlikely that they would occur in the absence of other major signs of severe disease; when present, these signs almost always denote underlying chronic hypertension*
- Pulmonary edema

Eclampsia is the occurrence of seizures in a patient with preeclampsia that cannot be attributed to other causes.

Preeclampsia superimposed on chronic hypertension. There is ample evidence that preeclampsia may occur in women already hypertensive (i.e., who have chronic hypertension), and the prognosis for mother and fetus is much worse than with either condition alone. The Committee on Terminology[8] recommended that the diagnosis be made on the basis of increases of blood pressure (30 mm Hg systolic or 15 mm Hg diastolic or 20 mm Hg mean arterial pressure) together with the appearance of proteinuria or generalized edema.

Transient hypertension. Transient hypertension is the development of elevated blood pressure during pregnancy or in the first 24 hours post partum without other signs of preeclampsia or preexisting hypertension. This condition is often predictive of the eventual development of essential hypertension. This is obviously a retrospective diagnosis, and if any uncertainty regarding the diagnosis exists, these patients should be managed as if they had preeclampsia.

Problems with classification. Problems are inherent in the use of blood pressures measured in early pregnancy to diagnose chronic hypertension or to define basal blood pressure for the diagnosis of preeclampsia. Blood pressure usually decreases early in pregnancy, reaching its nadir at about the stage of pregnancy at which women usually seek obstetric care. The decrease averages 7 to 10 mm Hg for diastolic readings. In some women, obviously, blood pressure decreases more than 7 to 10 mm Hg, and the early decline and subsequent return of blood pressure to or toward prepregnant levels in late gestation will be sufficient to diagnose preeclampsia. Because women who are hypertensive before pregnancy have a greater decrease in blood pressure in early pregnancy than do normotensive women,[12] they are even more likely to be erroneously diagnosed as having preeclampsia according to blood pressure criteria.

It is well established that seizures as a result of eclampsia may occur when the blood pressure is only mildly increased.[13, 14] Therefore any rise in blood pressure in a pregnant woman is potentially dangerous, and the final diagnosis of "mild" or "severe" preeclampsia should not be made until after the patient is delivered and returns home.

Even the triad of proteinuria, edema, and hyperten-

sion is nonspecific; each sign could be the result of conditions other than preeclampsia. Edema is a physiologic finding present in many normal pregnant women. There is no reason to believe that the normal physiologic edema of pregnancy should not occur in women with transient hypertension, but when it does the clinically oriented classification calls for the diagnosis of preeclampsia (almost always "mild preeclampsia"). Transient hypertension is sometimes the preproteinuric phase of preeclampsia and sometimes the recurrence of chronic hypertension abates in midpregnancy, but more often it seems to be a manifestation of latent essential hypertension brought to light by pregnancy, as several follow-up studies have shown.[15-17] Transient hypertension has a high rate of recurrence in later pregnancies (up to 88%) and probably is the usual basis for the erroneous diagnosis of preeclampsia in multiparous women.

The diagnosis of superimposed preeclampsia is especially difficult. Women fulfilling diagnostic criteria for superimposed preeclampsia may indeed have preeclampsia, but in some of these women the origin of signs may instead be renal disease, either causing the hypertension or caused by it, that may coincidentally worsen during pregnancy. Conversely, many women who actually have preeclampsia may not have been diagnosed. This is a special problem in women receiving antihypertensive therapy, in whom early stages of superimposed preeclampsia without proteinuria may be masked by therapeutic lowering of blood pressure. Superimposed preeclampsia is especially difficult to diagnose in the hypertensive woman with proteinuria at the onset of pregnancy. To aid in diagnosis, investigators have suggested using criteria such as increasing levels of serum uric acid and decreasing platelet counts.[18]

Preeclampsia has a clinical spectrum ranging from mild to severe forms and then potentially to eclampsia. Affected patients do not "catch" eclampsia but progress through this spectrum. In most cases progression will be slow and the disorder may never proceed beyond mild preeclampsia. In others the disease may progress more rapidly, changing from mild to severe in days to weeks. In the most serious cases, progression may be fulminant, with mild preeclampsia evolving to severe preeclampsia or eclampsia within days or even hours. *Thus for clinical management, preeclampsia should be overdiagnosed, because a major goal in managing preeclampsia is the prevention of eclampsia, primarily through timing of delivery.*

More stringent criteria must be used in the selection of cases for research studies of preeclampsia. No classification is accurate or verifiable in the absence of a good understanding of the cause of preeclampsia to permit the development of a specific diagnostic test. Definitions that depend on a numeric threshold arbitrarily imposed on a continuous spectrum of changes (e.g., rising blood pressure) create artifacts around the chosen threshold. This is particularly true of the definitions for hypertension in pregnancy and preeclampsia. A major obstacle to the advancement of our knowledge of preeclampsia-eclampsia is the publication of studies of "mild preeclampsia," because the diagnosis is likely to be erroneous in half the cases; the women may have preeclampsia, transient hypertension, or chronic hypertension that abated during much of pregnancy or any of a wide variety of renal diseases.[19]

Another problem pertinent to clinical management is that some women with pregnancy-specific pathophysiologic changes identical to those present in preeclampsia, including coagulation and liver function abnormalities, will not have the specific signs of preeclampsia sufficient to satisfy the ACOG definition.[20] These women are nonetheless at increased risk, as are their infants, and should be managed like the more typical patient with preeclampsia.

In spite of the difficulties in making the clinical diagnosis, there is no question that a pregnancy-specific condition (preeclampsia in the ACOG Committee's classification)[8] in which reduced perfusion to many organs, including the placenta, results in increased maternal and fetal morbidity and death. Unlike chronic hypertension, in which hypertension is the important pathogenic factor, in preeclampsia blood pressure is usually important primarily as a marker of disease. Also unlike chronic hypertension, which progresses slowly over the years, preeclampsia can proceed rapidly, with attendant increased risk to mother and infant. Thus it is mandatory to attempt to differentiate these disorders to appropriately manage the pregnant woman with increased blood pressure. *If a diagnosis of preeclampsia cannot be excluded, it is most appropriate to manage the woman as though she had preeclampsia.*

Blood pressure in normal pregnancy and the epidemiology of hypertensive disorders

As noted previously, diastolic pressure decreases by an average of about 7 to 10 mm Hg early in gestation, with a rise to or toward the (assumed) prepregnancy levels in the third trimester. Only minimal changes in the systolic pressure seem to occur, which is reasonable because the increase of 30% to 40% in the cardiac output largely offsets the decrease in total peripheral resistance.

The following factors predispose to preeclampsia-eclampsia:

- Primigravid women are from six to eight times more susceptible than are multiparous women.[21]
- About one third of primigravid women who have had eclampsia have hypertension in about 20% of

later pregnancies, but generally they have mere rises in blood pressure alone (transient hypertension) rather than preeclampsia. Only 10% to 15% have proteinuric hypertension.[1] Transient hypertension, usually miscalled mild preeclampsia in the past as well as in some current classifications, recurs in 80%[16] to 88%[15] of women pregnant again and often portends ultimate chronic hypertension.

- Multiparous women who have had preeclampsia or eclampsia usually have some predisposing factor, often chronic hypertension; in these women the recurrence of superimposed preeclampsia may be as high as 70%.[1]
- The tendency for preeclampsia is inherited.[22]
- Twin pregnancy increases the risk five times.[21]
- Diabetes is a potent factor, but relative risk cannot be specified because diabetic persons often have other forms of hypertension that confuse the diagnosis.[23]
- Large, rapidly growing hydatidiform moles increase the risk 10 times in both primigravid and multiparous women.[24]
- Fetal hydrops increases the risk 10 times in both primigravid and multiparous women.[25]

Relative risk of chronic hypertension cannot be estimated because the incidence of superimposed preeclampsia varies greatly as diagnosed and is greatly overdiagnosed from one center to another.

Contrary to popular belief, hydramnios alone[26] and social class[27] do not predispose to preeclampsia, which has the same incidence in white and black American women. Lack of prenatal care may lead to failure to recognize this condition in its early stages. Black women in the childbearing age have two to three times the prevalence of essential hypertension than white women. In black women frank and latent chronic hypertension and transient hypertension often are misdiagnosed as preeclampsia.

Pregnancy can be considered a screening test for ultimate chronic hypertension because women with normotensive pregnancies, especially after age 25 years, have a low likelihood of developing later chronic hypertension; women with transient hypertension have a high probability of ultimate essential hypertension. Preeclamptic and eclamptic hypertension predict nothing, because the disorder does not permit the screening. Had it not been for the intercurrent and unrelated preeclampsia, the pregnancy could have been either normotensive or complicated by transient hypertension.

The pathology and pathophysiology of hypertension in pregnancy focusing on preeclampsia

Vasospasm. Vasospasm is presumed basic to the pathophysiology of preeclampsia-eclampsia. Vascular constriction causes resistance to blood flow and subsequent arterial hypertension. In some presentations the cardiac output is not maintained, and the resulting absent or mild hypertension underestimates the severity of the disorder. In this context it should be noted that there is good evidence for the condition of "normotensive preeclampsia."[28] Vasospasm and associated vascular damage that cause endothelial cell leaks, together with local hypoxia of surrounding tissues, presumably lead to hemorrhage, necrosis, and other end-organ disturbances of severe preeclampsia.

Vascular reactivity to infused angiotensin II and catecholamines is decreased in normal pregnancy. There is an increased pressor response to some vasoactive hormones in early preeclampsia,[29] and this increased response clearly precedes the onset of hypertension.[30] Angiotensin II refractoriness, observed in normal pregnancy, may be mediated by vascular endothelial synthesis of vasodilatory prostaglandins (e.g., prostacyclin or prostaglandin E_2 or a prostaglandin-like substance). There are data suggestive that preeclampsia may be associated with inappropriately increased production of a prostaglandin with vasoconstrictor properties or either increased inactivation or diminished synthesis or release of another with vasodilator properties or a combination of these events.

Cardiovascular changes. Values obtained by invasive cardiovascular monitoring in women with severe preeclampsia and eclampsia have helped to define cardiovascular status. At least five general observations can be made[31-33]: (1) Before treatment, myocardial contractility is rarely impaired. (2) Cardiac afterload is elevated in the absence of therapeutic interventions. (3) Cardiac output varies inversely with vascular resistance. (4) Medications that reduce vascular resistance (e.g., hydralazine) result in increased cardiac output. (5) Ventricular preload, measured by central venous and pulmonary capillary wedge pressures, is usually normal or even low in severe preeclampsia and eclampsia, unless substantial volumes of fluids are administered.

Hemoconcentration is common in women with severe preeclampsia or eclampsia, and the intravascular volume expansion that is normal for pregnancy is not present or is reduced significantly.[34] The woman of average size usually has a blood volume of nearly 5000 ml during the last several weeks of a normal pregnancy, compared with about 3500 ml when nonpregnant. With preeclampsia or eclampsia, however, pregnancy-induced hypervolemia either never develops or is reduced significantly after vasospasm ensues. In the absence of hemorrhage, the intravascular compartment in these women usually is not "underfilled," because vasospasm has contracted the space to be filled. Hemoconcentration usually persists a few hours to a few days after delivery when typically the vascular system dilates,

the blood volume increases, and the hematocrit level falls. *In the woman with severe preeclampsia or eclampsia, therefore, hypertension may be exacerbated and the risk of pulmonary edema increased as the result of vigorous fluid therapy administered in an attempt to expand the contracted blood volume to normal pregnancy levels. Likewise hypovolemia can develop even with normal blood loss at delivery.*

Hematologic changes. Thrombocytopenia, although infrequently severe, is the most commonly found hematologic aberration (by routine clinical testing) associated with hypertension in pregnancy. Fibrin degradation products in serum are elevated only occasionally. Unless there is some degree of abruptio placentae, plasma fibrinogen levels do not differ remarkably from levels found late in normal pregnancy.[35]

Antithrombin III levels are lower and fibronectin levels higher in women with preeclampsia compared with normal pregnant women; these levels are consistent with those in vascular endothelial injury.[36, 37] The clinical utility of serial antithrombin III or fibronectin measurements for the diagnosis and management of preeclampsia awaits further evaluation.

The development of overt thrombocytopenia (i.e., a platelet count $<100,000/\mu l$) is an ominous sign in women with preeclampsia. Without delivery, the platelet count most often continues to decrease and may reach levels that can result in cerebral or subcapsular hepatic bleeding, as well as excessive blood loss during and after delivery, especially by cesarean section. *Maternal thrombocytopenia is not an indication for cesarean delivery.*

The cause of the thrombocytopenia is not firmly established. It has been ascribed to platelet deposition at sites of endothelial damage[35] or to an immunologic process.[38] There is no firm evidence that the fetuses-infants born to women with severe preeclampsia-eclampsia will have thrombocytopenia, despite severe maternal thrombocytopenia.[39]

Renal changes. The majority of women with preeclampsia have mild to moderately diminished renal perfusion and glomerular filtration with correspondingly elevated plasma creatinine and uric acid levels. An elevated plasma creatinine level is a late development in the evolution of preeclampsia and is often discerned only in the range that would be considered normal for nonpregnant individuals (e.g., 0.8 to 1.0 mg/dl). In some cases of severe preeclampsia, renal involvement may be profound and plasma creatinine levels may be elevated twofold to threefold over normal values in nonpregnant women. This probably is caused by intrinsic renal changes.[40] In unusual instances, preeclampsia may lead to acute tubular, and even cortical, necrosis.

Although some degree of proteinuria should be found for the diagnosis of preeclampsia-eclampsia to be reliable,[41] proteinuria usually develops late in the course of the disease, so some women may be delivered before it appears. Thus they may have "true preeclampsia" without proteinuria. This type of proteinuria is nonselective; the increased permeability includes proportionally more of larger molecular weight proteins such as transferrin and several globulins than in renal diseases with selective proteinuria. Thus abnormal albumin excretion is accompanied by increased quantities of many other proteins. Proteinuria usually recedes within 1 week after delivery and resolution of hypertension, but in exceptional cases the protein leak may take more than 1 month to heal.

Renal changes identifiable by light and electron microscopy include those in the capillary loops, which are variably dilated and contracted. The endothelial cells are swollen; deposited within and beneath these cells are fibrils, which have been mistaken for thickening and reduplication of the basement membrane. Electron microscopic studies are consistent with the view that the characteristic changes are caused by swelling of *intraglomerular* cells, primarily endothelial cells but on occasion mesangial cells. These changes, which may be accompanied by subendothelial deposits of a fibrinlike protein material, are termed *glomerular capillary endotheliosis*.[40, 42]

Hepatic changes. Severe preeclampsia may result in alterations in tests of hepatic function and integrity, including elevation of serum aspartate aminotransferase levels. The lesion most likely to account for hepatic impairment is *periportal hemorrhagic necrosis*. Bleeding from these lesions may extend beneath the hepatic capsule to form a *subcapsular hematoma*. Hepatic involvement in preeclampsia-eclampsia is serious, and it frequently is accompanied by evidence of involvement of other organs, especially the kidney and brain, along with hemolysis and thrombocytopenia.[43]

Changes in the brain. The principal postmortem lesions described in the brains of women who died of eclampsia are hyperemia, focal anemia, thrombosis, and hemorrhage.[44] Cerebral blood flow, oxygen consumption, and vascular resistance were reported as not altered in women with preeclampsia,[45] but the possibility of focal blood flow changes could not be excluded. However, cerebral oxygen consumption is reported to be decreased by 20% in eclampsia with normal blood flow, such that McCall[45] alluded to it as "histotoxic hypoxia." Nonspecific abnormalities in the electroencephalogram are common in women with eclampsia within 48 hours of seizures, but the tracings are usually normal by 3 months.[46]

Until recently, cranial computed tomographic scans usually were reported to be normal in women with

otherwise uncomplicated eclampsia. However, with more advanced equipment, nearly half of women with eclampsia have abnormal radiographic findings.[47] The most common of these findings are hypodense areas, frequently seen in the cortical areas, that correspond to areas of petechial hemorrhage and infarction.[48]

Although visual disturbances are common with severe preeclampsia, blindness, either alone or accompanying convulsions, is uncommon. Women with *amaurosis* of varying degrees usually have computerized tomographic evidence of extensive occipital lobe hypodensities. These women completely recover within 1 week. *Retinal detachment* also may cause altered vision, although it is usually one-sided and seldom causes total loss of vision. Even without surgical treatment, vision usually returns to normal within approximately 1 week.

Uteroplacental perfusion. Compromised placental perfusion caused by placental disease (failure of trophoblastic invasion of spiral arteries) and vasospasm is almost certainly a major culprit in the genesis of increased perinatal morbidity and death associated with preeclampsia. However, there are formidable problems to measuring uteroplacental blood flow in humans with preeclampsia. In an earlier study[49] it was observed that ^{24}Na injected into the intervillous space was cleared two to three times more rapidly in normotensive pregnant women than in women with preeclampsia, implying decreased uteroplacental perfusion.

These findings are supported indirectly by studies of the clearance rate of dehydroisoandrosterone sulfate through placental conversion to estradiol-17β. More recently, measurement of the velocity of blood flowing through the uterine arteries has been used to estimate uteroplacental blood flow. Using arterial velocity waveforms obtained by Doppler ultrasonography, vascular resistance is estimated by comparing systolic and diastolic waveforms. Normally the uterine vascular bed is a low-resistance circuit and flow continues throughout diastole. As resistance increases, diastolic velocity diminishes in relation to systolic velocity, and this relationship is used to estimate decreased flow. Some investigators report an increased systolic-diastolic ratio in uterine arteries of women with preeclampsia.[50, 51] It should be emphasized that the aforementioned methods do not measure perfusion.

Differential diagnosis of hypertension in pregnancy

Discussions concerning the clinical spectrum of the hypertensive disorders in pregnancy relate to those symptoms, signs, and laboratory aberrations that permit differentiation of preeclampsia (pure or superimposed) from the more benign forms of high blood pressure, mainly essential and gestational (or transient) hypertension.

The need to distinguish preeclampsia from other disorders is particularly important in the management of women in midpregnancy (before gestational week 28) and in counseling about future pregnancies. With respect to the former, patients with midterm elevations in blood pressure caused by essential hypertension may be managed conservatively even when they have moderate to severe degrees of hypertension. However, attempts to temporize in the presence of severe preeclampsia can be disastrous, as shown by Sibai et al.[52] These authors attempted conservative therapy in 60 consecutive gravida women with severe preeclampsia between the eighteenth and twenty-seventh gestational weeks. The perinatal mortality rate was 87%. Ten mothers had convulsions, two had hypertensive encephalopathy, and one had an intracerebral hemorrhage. In addition, there were five instances of severe coagulopathy, one resulting in a ruptured capsular hematoma of the liver, and three women had acute renal failure. Although there are still dissenting opinions, most authorities consider temporization of *severe* midterm preeclampsia too risky, and interruption of gestation is usually in the mother's best interest.

The second reason for establishing a correct diagnosis relates to counseling. Subsequent pregnancies are often apt to be normotensive when preeclampsia-eclampsia develops in nulliparous women, whereas preeclampsia (or high blood pressure as a result of other causes) in a multiparous woman is associated with a greater likelihood of hypertension in subsequent gestations. In summary, when hypertension complicates pregnancy, establishing an accurate diagnosis may be useful both to help clinicians assess the immediate well-being of mother and fetus and to counsel her when she seeks advice on future pregnancies.

Results from renal biopsy studies underscore the difficulty in distinguishing clinically between preeclampsia (pure or superimposed) and other causes of high blood pressure in pregnancy. Investigators continue to seek sensitive and specific tests with which to make this differentiation.[1, 19] Some tests now of historic interest only include demonstration of decreased fractional urate clearance, reduced sodium excretory capacity after infusions of hypertonic saline solution, and exaggerated pressor responses to vasopressin or after changing from lateral recumbency to a supine posture (the "roll-over" maneuver). These tests have proved unreliable or have insufficient specificity to make them clinically useful.[1]

Several additional approaches that might help in the correct diagnosis of pure or superimposed preeclampsia have been proposed recently. These include mea-

surements of plasma antithrombin III,[53] serum iron and carboxyhemoglobin,[54] and urinary excretion of calcium.[55] Currently, however, the validity of these approaches has not been proved,* and some have been challenged.[58] Other tests under study are detection of antibodies to laminin[57] and identification of serum factors that increase mitogenic activity in fibroblasts or are toxic to endothelial cells in culture.[57, 58]

Recommended laboratory tests. Laboratory tests may aid in the diagnosis and management of hypertension during pregnancy. They also provide reference data when high-risk patients are first seen for prenatal care.

High-risk patients with normal blood pressure. Gestations in women with normal blood pressure but at high risk include women with a history of increased blood pressure when not pregnant, those who have had hypertension in a previous pregnancy other than the first, and those with diabetes, collagen vascular disease, or underlying renal vascular or renal parenchymal disease (see section on Blood Pressure in Normal Pregnancy and the Epidemiology of Hypertensive Disorders). Certain baseline data, when compared with results obtained in later gestations, will assist in the early diagnosis of preeclampsia. Baseline data in such women should include hematocrit, hemoglobin, and platelet count, as well as serum creatinine and uric acid levels. If routine urinalysis demonstrates qualitative proteinuria of 1+ or greater, a 24-hour collection should be obtained, including determination of creatinine content to check for accuracy of measurement and to permit calculation of the creatinine clearance. Some might include serum albumin and serum oxaloacetic transaminase determinations in the baseline assessment, whereas others would seek these tests only in the presence of frank hypertension. These patients should also undergo ultrasonography at the initial visit for accurate dating and as a baseline for evaluating subsequent fetal growth.

Patients with hypertension before gestational week 20. Most women with hypertension before gestational week 20 have (or will have) essential hypertension, and their management is discussed in the next section. Some of these patients will be under the care of primary physicians and will have been screened for signs of secondary hypertension. If not, we recommend the approach to evaluation and diagnosis of hypertension suggested in The 1988 Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure.[7] Finally, gravid women with preexisting or early gestational hypertension are among the population in whom secondary hypertension is more apt to be found (e.g., renal disease, renovascular hypertension, primary aldosteronism, Cushing's syndrome, and pheochromocytoma). Thus further evaluation is warranted even for minimal suspicion. For example, pheochromocytoma, although rare, has a propensity to manifest or be activated by pregnancy, and it is associated with high maternal mortality rates when undiagnosed.[60, 61] Thus suspicion warrants performance of a screening test to detect pheochromocytoma (e.g., 24-hour excretion rate of vanillylmandelic acid or metanephrines or plasma norepinephrine and epinephrine levels).

Baseline determinations of renal function (serum creatinine and uric acid levels) and platelet count can be compared with values in later pregnancy to help determine if increases in blood pressure at this time are the usual physiologic increases in blood pressure or the onset of preeclampsia. Because these fetuses are at high risk for the development of intrauterine growth retardation, baseline ultrasonography for dating and fetal size are indicated before 20 weeks' gestation.

Patients with hypertension after midpregnancy. Table I summarizes the laboratory evaluation of women with hypertension after midpregnancy and the rationale for testing them biweekly or more often if clinical circumstances lead to hospitalization of the patient. It is important to recognize that one or more of these abnormalities may be present even when blood pressure elevation is minimal. If there is a life-threatening abnormality such as coagulopathy or abnormal hepatic or renal function, it may be necessary to terminate the pregnancy despite only mild hypertension (see section of Management of Preeclampsia).

Management of hypertension in pregnancy

Management of preexisting chronic hypertension in pregnancy

Counseling. Hypertensive women considering pregnancy should be counseled before conception. They should be informed of the high likelihood of a favorable outcome in most cases of mild to moderate essential hypertension. They should be aware of the increased risk of superimposed preeclampsia and the possible complications if preexisting renal disease or systemic illness is present.

It is helpful to inform women before pregnancy of possible adjustments in life-style that may be necessary during pregnancy if the blood pressure is elevated, specifically, the possibility that restricted activity, bed rest, or even hospitalization may be advisable.

Patients with intrinsic renal disease who are normotensive and have minimal renal dysfunction usually do well in pregnancy. However, those with azotemia (creatinine >2 mg/dl or, some say, 1.5 mg/dl) and hypertension should be advised of a number of risks.[62, 63]

*Studies designed to detect preeclampsia are currently hampered by the fact that renal biopsies are rarely indicated today, and absence of morphologic correlations makes it difficult to assess the true sensitivity and specificity of test results.

These include a high incidence of superimposed preeclampsia, increased risk of perinatal morbidity and death, and the possibility that maternal renal function may deteriorate further as a result of the pregnancy.[63, 64] These concerns are especially applicable to women with renal transplants, in whom pregnancy should be undertaken only after consultation with a nephrologist with expertise in this area.

Nonpharmacologic treatment. Close medical supervision is the mainstay of management of pregnant women with chronic hypertension. It is preferable to control the blood pressure without medication when possible. In patients with diastolic blood pressure from 90 to 99 mm Hg, this goal is not difficult to attain, because blood pressure falls in most pregnant women during the first and second trimesters.

The strategies for nonpharmacologic treatment of hypertension during pregnancy differ from those in nonpregnant individuals. Whereas weight reduction and exercise might benefit a nonpregnant individual, these measures are not encouraged during pregnancy. Although there are no studies that address the issue of exercise in hypertensive pregnancy, given the theoretic concerns regarding the role of the uteroplacental blood flow in the pathogenesis of preeclampsia, women with chronic hypertension should not be encouraged to participate in vigorous exercise.

Restriction of activity. Bed rest is a good means of maximizing uteroplacental blood flow during pregnancy and is considered established therapy in preeclampsia. In chronic hypertension its effectiveness has not been well studied, but it is an integral feature of management. Rest has been shown to reduce premature labor, lower blood pressure, and promote diuresis.[65] Strict bed rest is rarely necessary. However, all pregnant women with elevated blood pressure, whatever the cause, should be advised to limit their activities when possible and set aside time during the day when they can be "off their feet." Although many women may find it difficult to restrict their activity, it is helpful to explain the benefits to the patient before pregnancy so she can make adjustments in child care, her job, and other responsibilities. In patients with mild to moderate hypertension, restriction of activity may be effective in lowering blood pressure and antihypertensive medications may be avoided. It is usually advisable to hospitalize patients with severe hypertension for bed rest and medication when necessary.

Diet. Weight reduction may be helpful in reducing blood pressure in nonpregnant individuals, but it cannot be recommended during pregnancy. If a hypertensive woman is overweight and planning a pregnancy, weight reduction before pregnancy is advisable.

Pregnant women with hypertension have lower plasma volume than do normotensive women, and some studies suggest that the severity of hypertension correlates with the degree of plasma volume contraction.[66] For this reason, sodium restriction is generally not recommended during pregnancy.[67] If, however, a pregnant women with chronic hypertension is known to have salt-sensitive hypertension and has been treated successfully with a low-salt diet before pregnancy, it is reasonable to continue some sodium restriction during pregnancy. Patients with renal disease and reduced creatinine clearances are more likely to require sodium restriction for blood pressure control, and this should be continued during pregnancy. The amounts of weight gained during the pregnancy may be an indication of whether salt intake is appropriate.

Preliminary studies have shown that dietary calcium supplementation lowers blood pressure in pregnant and nonpregnant individuals.[68, 69] However, there are insufficient data to recommend its use for treatment of hypertension.

Table I. Laboratory evaluation of women who develop hypertension after midpregnancy

Test	*Rationale*
Hemoglobin and hematocrit	Hemoconcentration supports diagnosis of preeclampsia and is an indicator of severity. Values may be decreased, however, if hemolysis accompanies the disease.
Blood smear	Signs of microangiopathic hemolytic anemia (e.g., schistocytes) favor diagnosis of preeclampsia and may be present when blood pressure is only mildly elevated.
Platelet count	Decreased levels suggest severe preeclampsia.
Urinalysis	If qualitative dipstick is 1+ or greater, a quantitative measurement of protein excretion should be done. Hypertensive gravid women with proteinuria should be considered to have preeclampsia (pure or superimposed) until proved otherwise.
Serum creatinine level	Abnormal or rising levels, especially when assoicated with oliguria, suggest severe preeclampsia.
Serum uric acid level	Increased levels aid in the differential diagnosis of preeclampsia and are an indicator of disease severity.
Serum oxaloacetic transaminase	Abnormal values suggest severe preeclampsia with hepatic involvement.
Lactic acid dehydrogenase	Elevated levels are associated with both hemolysis and hepatic involvement and suggest severe preeclampsia.
Serum albumin	Values may be decreased even in the absence of heavy proteinuria and may relate to "capillary leak" or hepatic involvement in preeclampsia.

Home blood pressure monitoring. The rationale for close observation of the pregnant woman with hypertension is that changes in clinical status (such as development of superimposed preeclampsia) can be recognized before they become severe. This is easier to accomplish if patients are instructed to take their blood pressures at home and keep a record of the readings. They can then contact medical personnel if the blood pressure rises significantly before a scheduled visit. Home monitoring of blood pressure is often helpful because it will enable the patient to determine how much limitation of activity is necessary to keep her blood pressure controlled. This technique also helps discriminate truly elevated blood pressure from labile blood pressure increases common in young patients. Patients who are candidates for home blood pressure monitoring should have formal instruction on the correct technique for determination of blood pressure. In addition, their equipment should be checked periodically for accuracy. Preliminary data on home blood pressure monitoring during pregnancy suggest that it is a useful adjunct to the care of the pregnant woman.[70]

Alcohol and tobacco. The use of alcohol and tobacco during pregnancy should be discouraged strongly. Both have a deleterious effect on the fetus and mother. Excessive consumption of alcohol can aggravate hypertension or raise blood pressure in the mother de novo.

Rationale for pharmacologic treatment. The majority of women with chronic hypertension in pregnancy have mild to moderate elevations in blood pressure, and therefore the risk of acute cardiovascular complications is extremely low. In the small percentage of women who have severe hypertension, the increased risk of cerebral hemorrhage, cardiac failure, and myocardial infarction necessitates close monitoring and aggressive treatment during pregnancy.

Although there is increased risk of perinatal morbidity and death when the mother has chronic hypertension,[1, 71, 72] most pregnancies in these women result in healthy, full-term infants. Women with chronic hypertension are at increased risk of development of superimposed preeclampsia, and there is evidence that most, if not all, of the increased perinatal morbidity and death associated with chronic hypertension is attributable to this complication.[73] However, the literature is conflicting. Some studies have shown that infants born to women with chronic hypertension in pregnancy who do *not* have superimposed preeclampsia do as well as those born to normotensive women.[73] Other studies report a higher perinatal loss in uncomplicated pregnancy in hypertensive women (compared with normotensive women), especially with higher levels of blood pressure.[1]

The objective in treating a pregnant woman with chronic hypertension is to minimize the short-term risks to the mother of elevated blood pressure while avoiding therapeutic maneuvers that compromise fetal well-being. The specific goals for the mother are to prevent cardiovascular complications of severe hypertension and, if possible, preeclampsia. When maternal blood pressure reaches diastolic levels of 100 mm Hg or more, treatment should be instituted to avoid hypertensive vascular damage. Some experts believe at least nonpharmacologic treatment is warranted at diastolic blood pressures of 90 mm Hg or greater.

The indications for treatment of hypertension (at diastolic blood pressures of 90 to 99 mm Hg) during pregnancy are less clear. In the nonpregnant individual, treatment of mild to moderate hypertension is recommended for prevention of long-term cardiovascular consequences of elevated blood pressure.[7]

To date, clinical trials and clinical experience have not given us a conclusive answer to the question, "Does treatment of chronic hypertension prevent preeclampsia?" Many of the clinical trials of antihypertensive therapy in chronic hypertension have evaluated treatment begun in the third trimester.[74-76] This is largely a consequence of the fact that blood pressure falls in most women during the first trimester, reaching its nadir by midpregnancy. This phenomenon occurs in patients with chronic hypertension as well, and because most patients have only mild hypertension to begin with, by midpregnancy the majority will be normotensive. Therefore it is frequently not until the third trimester, when the blood pressure rises, that treatment is begun. If superimposed preeclampsia in the patient with chronic hypertension is associated with the same placental disease as in pure preeclampsia, then the definitive morphologic changes are already present by 20 weeks. Thus, one would not expect antihypertensive medication begun in the third trimester to alter these changes. To complicate matters, patients enrolled in clinical trials that have started treatment in the third trimester are frequently a heterogeneous population and include patients with transient hypertension, preeclampsia, and chronic hypertension.

There are almost no data available that would either support or dispute the notion that early treatment of chronic hypertension (in the first half of pregnancy) prevents superimposed preeclampsia. Carefully conducted clinical trials are needed to resolve this issue.

It is not uncommon for a pregnant woman with chronic hypertension to be hospitalized because of elevated blood pressure. Early treatment of hypertension in pregnancy may reduce the need for such hospitalization, but it may obscure the diagnosis of superimposed preeclampsia, because a rise in blood pressure may be the first sign of this condition.[77] It is particularly

important to monitor women being treated for hypertension during pregnancy for signs of preeclampsia, because this condition is associated with adverse fetal outcome.

With regard to fetal well-being, several studies suggest, but do not conclusively demonstrate, benefits of treating mild to moderate hypertension during pregnancy.[78] In the largest published clinical trial demonstrating a reduction in perinatal deaths in mothers treated with methyldopa, the major intake of patients was during the second trimester, with a significant proportion entering earlier in the first half of pregnancy.[79] Because the beneficial outcome was the result of a decrease in midtrimester pregnancy losses, it is not clear whether lowering blood pressure was responsible for the improved outcome. Given the potential hazards of antihypertensive treatment during pregnancy (the possibility that medication may reduce placental blood flow or adversely affect the fetus), treatment of hypertension (at diastolic levels of 90 to 99 mm Hg) must be undertaken cautiously, weighing the risks and benefits of treatment for both mother and child at all times. Excessive reduction in blood pressure is to be avoided, and a conservative approach is recommended.

Drug treatment of chronic preexisting hypertension. The goal of treating chronic hypertension during pregnancy is the reduction of maternal risk and perhaps that of the fetus (see section on Management of Preexisting Chronic Hypertension in Pregnancy). With this goal in mind it is mandatory to establish not only the efficacy of drugs to reduce blood pressure but also the acute and long-range effects of these drugs on fetal well-being, especially (based on the mechanism of action of many antihypertensive drugs) long-range neurologic effects. So far only one drug, methyldopa, meets these criteria. Thus if feasible, methyldopa therapy should be chosen in pregnancy. If this drug is ineffective or cannot be tolerated (which happens not infrequently), alternative therapy is determined by guidelines based on limited clinical experience and rational choices based on the mechanisms by which the drugs act. It is important to point out that, in spite of theoretic concerns, none of the drugs currently used to treat hypertension other than the angiotensin-converting enzyme inhibitors have been demonstrated to increase the risk in perinatal morbidity or death within the limits of acute follow-up. The following categories of antihypertensive agents are addressed alphabetically.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS. This new class of antihypertensive agents, which act in part by inhibiting conversion of angiotensin I to angiotensin II, has become popular in the treatment of essential hypertension and hypertension associated with renal disease in nonpregnant patients because of its minimal side effects. Although no teratogenic effects have been observed in animals using these drugs, they do lower uterine blood flow and reduce fetal survival in pregnant rabbits and sheep, perhaps by decreasing uterine prostaglandin E_2 and I_2 synthesis.[80] Acute renal failure with lethal consequences has also been described in the neonates of women treated with angiotensin-converting enzyme inhibitors in the last trimester.[81-84] Because other antihypertensive agents are available, it is recommended that angiotensin-converting enzyme inhibitors be avoided during pregnancy.

β-ADRENERGIC BLOCKING AGENTS. Evidence is accumulating that β-adrenergic blocking drugs are useful and sufficiently safe in treating preexisting hypertension during pregnancy.[78, 85] There is also evidence that the combined α- and β-blocking agent labetalol (α and β antagonism ratio 1:4) is relatively safe and effective in pregnancy.[85-88] Most studies to date demonstrate equal effectiveness when β-adrenoreceptor antagonists and the combined α- and β-blocker labetalol are compared with methyldopa.[85]

Finally, despite a growing and reassuring literature of controlled trials with β- and combined β- and α-adrenoreceptor agents, we must interject a note of caution. Most studies were relatively small and gestational age at entry was usually 29 to 33 weeks, leaving unanswered the possibility that treatment of larger numbers of patients or a longer duration of drug administration may reveal adverse effects.[87, 89] For instance, Butters et al.[89] suggest that the long-term use of β-blockers predisposes to major growth retardation. Finally, although some prefer β-adrenergic blockers because they cause less somnolence than methyldopa, others continue to favor the α_2-agonists because of concern that β-blocking agents, which cross the placenta, may interfere with interpretation of fetal heart rate, as well as because of the theoretic possibility that β-blockers compromise the ability of the fetus to withstand hypoxic stress.

CALCIUM CHANNEL BLOCKERS. Calcium channel blockers have been used to treat essential hypertension for many years in Europe and recently have been introduced into the United States. Nifedipine, which has a greater effect on vascular smooth muscle than on the myocardium, has proved promising in treating hypertension. However, it is teratogenic in rats when given in doses 30 times the maximum recommended human dose. There are very few studies of its use throughout human pregnancy, although these drugs are used in later pregnancy as treatment for preterm labor, without adverse consequences.[90, 91]

CENTRALLY ACTING ADRENERGIC INHIBITORS. Methyldopa and clonidine are the two most commonly used central adrenergic antagonists and both are used during pregnancy.[92-94] Clonidine is a centrally acting adrenergic inhibitor that inhibits sympathetic output from

the central nervous system. The effect of methyldopa is more complex, but it also inhibits central sympathetic discharge. Methyldopa was the first antihypertensive agent used in the treatment of hypertension during pregnancy, so the experience with it is the longest. Follow-up studies of children born to mothers taking methyldopa throughout pregnancy have revealed normal mental and physical development in these children at 10 years of age.[95] Methyldopa given to women with essential hypertension has been demonstrated to reduce the number of midtrimester abortions without affecting neonatal survival or fetal growth.[79]

Methyldopa causes somnolence in many individuals. However, its demonstrated safety for the fetus in long-term follow-up[95] makes this the initial drug of choice in the management of chronic hypertension in pregnant women and the benchmark against which other antihypertensive agents must be tested.

DIURETICS. The use of diuretic agents in pregnancy is controversial. The primary concern is theoretic. It is known that preeclampsia is associated with a reduction of plasma volume,[95] and fetal outcome is worse in women with chronic hypertension who do not have expanded plasma volume.[97] Whether this is a cause-and-effect relationship is not clearly established. Nonetheless, women who use diuretics from early pregnancy do not have increased blood volume to the degree that usually occurs in normal pregnancy.[98] This theoretic concern must be tempered by extensive experience in several well-controlled studies with the prophylactic use of diuretics in normotensive gravid women, in whom no excess of perinatal death or morbidity was evident.[99] A metaanalysis of nine randomized trials comprised of more than 7000 subjects revealed a decrease in the tendency of these women to have edema or hypertension[100] and confirmed no increased incidence of adverse fetal effects. Based on the theoretic concerns, diuretics are usually not used as first-line drugs. However, if their use is indicated, they are safe and efficacious agents, can markedly potentiate the response to other antihypertensive agents, and are not contraindicated in pregnancy except in settings in which uteroplacental perfusion is already reduced (preeclampsia and intrauterine growth retardation).

Although data concerning the use of diuretics in pregnant women with essential hypertension are sparse, this Committee concluded that gestation does not preclude use of saluretic drugs to reduce or control blood pressure in women whose hypertension predated conception or manifested before midpregnancy. (The Joint National Committee IV recommended smaller doses of diuretics than were used previously, thus minimizing metabolic effects.[7])

VASODILATORS. Hydralazine is a vasodilator that is relatively ineffective when used alone because of the reflex tachycardia with increased cardiac output that occurs with its use. However, when hydralazine is combined with a β-adrenergic blocking agent, the reflex tachycardia is prevented, and the combination is quite effective in reducing blood pressure. Hydralazine is used extensively, usually with methyldopa, in treating preexisting hypertension in pregnancy and is considered to be safe for mother and fetus by most obstetricians. Still, one survey in Scandinavia has reported fetal thrombocytopenia.[101] Therefore its use as a first-line drug in treating pregnant women with chronic preexisting hypertension should be limited.

In summary, although this working group endorses judicious antihypertensive therapy for pregnant women with preexisting essential (chronic) hypertension (diastolic blood pressures greater than or equal to 90 mm Hg), there is no good existing evidence that the use of antihypertensive therapy improves fetal survival. Methyldopa may prevent midgestational losses.

Maternal-fetal surveillance. Whether the patient has acute or chronic hypertension, fetal surveillance is indicated. The methods for fetal surveillance are the same in both cases and the interpretation is similar. If the fetus is compromised, decision making and judgment are necessary. If intrauterine growth retardation is identified by fundal measurements and documented by ultrasonography, biophysical testing should be implemented. The amount of amniotic fluid is significant, because a decreased amount of fluid may be associated with cord problems during labor.

Nonstress testing, oxytocin challenge testing, ultrasonography, and fetal movement counts constitute the most common fetal surveillance techniques. If determination of pulmonary maturity would influence management, amniocentesis should be done to determine this before the interruption of pregnancy.

Fetal surveillance involves determining if the fetus is growing appropriately. When fundal height measurements are inappropriate, other investigative avenues such as ultrasound biophysical testing should be pursued. As long as the fetus continues to grow in an appropriate manner, it can be inferred that the placenta and uterine blood flow is appropriate.

Management of preeclampsia

Prevention of preeclampsia. Our ability to prevent preeclampsia is limited by lack of knowledge regarding its cause. The cornerstone of "prevention" in patients with preeclampsia has been identification of the high-risk individual (see section on Blood Pressure in Normal Pregnancy and the Epidemiology of Hypertensive Disorders), followed by close clinical and laboratory monitoring so that the disease process can be recognized in its early stages. Women can then be hospitalized for more intensive monitoring or delivery. Although these measures do not enable us to prevent preeclampsia,

they do help to prevent its catastrophic maternal and fetal sequelae.

Although various dietary and pharmacologic strategies (low-salt and high- or low-protein diet and diuretics) have been employed with the hope of preventing preeclampsia or minimizing its severity, none have proved effective so far. Many obstetricians consider daily rest to be effective in preventing preeclampsia or minimizing its severity in high-risk individuals, although this has not been proved.

One preventive strategy that is currently receiving a great deal of attention is the use of low-dose aspirin. At the present time many centers around the world are investigating the ability of low-dose aspirin to prevent preeclampsia. These clinical trials have been prompted by two initial encouraging reports.[102, 103] In both trials, low-dose aspirin[103] or low-dose aspirin plus dipyridamole[102] was successful in reducing the incidence of preeclampsia in the groups studied. There were no fetal or maternal complications attributable to aspirin in either study; however, the total number of women who received aspirin was fewer than 100. Two recently published trials of low-dose aspirin in moderate- and high-risk pregnant women support these earlier encouraging reports and provide evidence that the beneficial effects of aspirin are associated with selective inhibition of platelet thromboxane A_2 generation, with preservation of vascular prostacyclin generation.[104, 105] However, because aspirin in large doses is associated with hemorrhagic complications in the newborn,[106] and prostaglandins play a major role in maternal-fetal physiology, we do not recommend treatment of women at risk for preeclampsia with low-dose aspirin until large clinical trials have conclusively documented the safety and efficacy of this therapy.

Rationale for treatment. The objectives of therapy for preeclampsia are based on a philosophy of management arising from the knowledge of the pathology and pathophysiology and the prognosis of the disorder for mother and infant.

- **Delivery is always appropriate therapy for the mother but may not be so for the fetus.** For maternal health, the goal of therapy is to prevent eclampsia, *as well as other severe complications of preeclampsia.* Preeclampsia is the precursor of eclampsia, and careful antepartum observation can identify the woman at risk. Preeclampsia is completely reversible and begins to abate with delivery. Thus if only maternal well-being were considered, the delivery of all women with preeclampsia, regardless of severity of the process or stage of gestation, would be appropriate. Considering the fetus, however, one should not induce delivery in mildly preeclamptic women whose fetuses are immature but have no signs of fetal compromise. There are two important corollaries of this statement. First, any therapy for preeclampsia other than delivery must have as its successful end point the reduction of perinatal death and morbidity. Second, the cornerstone of obstetric management of preeclampsia is based on a decision as to whether the infant is more likely to survive in utero or in the nursery.
- **The signs and symptoms of preeclampsia are not of pathogenetic importance.** The pathologic and pathophysiologic changes of preeclampsia indicate that poor perfusion is the major factor leading to the derangement of maternal physiologic function and to increased perinatal mortality and morbidity rates.

Attempts to treat preeclampsia by natriuresis or the lowering of blood pressure do not alleviate the important pathophysiologic changes. In fact, natriuresis may be counterproductive and may adversely affect fetal outcome, because plasma volume is already reduced in women with preeclampsia.

- **The pathogenetic changes of preeclampsia are present long before clinical criteria leading to the diagnosis are manifest.** Several studies indicate that changes in vascular reactivity, plasma volume, and renal function antedate, in some cases by months, the increases in blood pressure, protein excretion, and sodium retention. These findings suggest that irreversible changes affecting fetal well-being may be present before the clinical diagnosis. This possibility probably explains why dietary, pharmacologic, and postural therapy is not successful when avoidance of perinatal morbidity and death is taken as the end point. If a rationale for modes of therapy other than delivery of the fetus exists, it would be to palliate the maternal condition to allow fetal maturation. However, even this rationale is controversial.

Accelerated and severe hypertension can complicate any phase of gestation. Fortunately, most of these crises occur late in pregnancy and are associated with preeclampsia; the challenge to the physician is to maintain maternal blood pressure at safe levels while effecting a delivery.

Nonpharmacologic management

MATERNAL EVALUATION. Antepartum monitoring of the mother has two goals. The first is the early recognition of the condition, because infants of mothers with even mild preeclampsia are at increased risk, and the second is to gauge the rate of progression of the condition, both to prevent eclampsia by delivery and to determine whether fetal well-being can be monitored safely by the usual intermittent observations. Ideally, identification of early changes would allow intervention before the advent of clinical symptoms. At present,

other than the early increased sensitivity to angiotensin II, which is not practical for application to widespread screening, no test has a predictive value sufficient to use clinically. Notably unsuccessful are the "rollover test" and elevated second-trimester blood pressures.

At present, clinical management is dictated by the overt clinical signs of preeclampsia. Unfortunately, proteinuria, the most valid clinical indicator of preeclampsia, is often a late change, sometimes even preceded by seizures, and so is not a useful sign for early recognition. Although rapid weight increase and facial and digital edema indicate the fluid and sodium retention characteristic of preeclampsia, they are neither universally present nor uniquely characteristic of preeclampsia. These signs are, at most, a reason for closer observation of blood pressure and monitoring of urinary protein levels. Early recognition of preeclampsia is based primarily on diagnostic blood pressure increases in the late second and early third trimesters relative to early pregnancy. Use of blood pressure changes without evidence of proteinuria as an indicator does, undoubtedly, result in the diagnosis of preeclampsia in some normal women, as well as in some with underlying renal or vascular disease. Because the goal of early diagnosis is to identify patients requiring more careful observation, however, overdiagnosis is preferable to underdiagnosis.

Once the blood pressure changes suggestive of preeclampsia appear, an office examination within 24 to 48 hours is strongly recommended or, with selected patients, blood pressure and urinary protein values must be checked at home. These measures are directed at determining how fast the condition is progressing to ensure that it is not following a fulminant course. Frequency of subsequent observations is determined by these initial observations and the ensuing clinical progression. If the condition appears stable, weekly observations may be appropriate. If it appears to be accelerating, more frequent observations, usually in the hospital, are required. The initial appearance of proteinuria is an especially important sign of progression and dictates frequent observations, which is best accomplished in the hospital.

If an increasing rate of deterioration is noted, as determined by laboratory findings, symptoms, and clinical signs, the decision to continue the pregnancy is determined day by day. Important clinical signs are blood pressure, urinary output, and fluid retention as evidenced by daily weight increase. Laboratory studies are performed at intervals of no more than 48 hours. These include examination for possible activation of the coagulation system as determined by platelet count and evaluation of renal function as measured by urinary protein excretion and serum creatinine and urate levels. In addition, subjective evidence of central nervous system involvement, such as headache, disorientation, and visual symptoms, and the presence of hepatic distention, as indicated by abdominal pain and hepatic tenderness, are equally important indicators of worsening preeclampsia.

ANTEPARTUM MANAGEMENT OF PREECLAMPSIA. There is little to suggest that therapeutic efforts alter the underlying pathophysiology of preeclampsia. Therapeutic intervention is palliative. At best it may slow the progression of the condition, but more likely it merely allows continuation of the pregnancy. Bed rest is a usual and reasonable recommendation for the woman with mild preeclampsia, although its efficacy is not clearly established. Strict sodium restriction or diuretic therapy has no role in the prevention or treatment of preeclampsia. In women with marked sodium retention as manifested by significant edema, modest sodium restriction may not alter the course of the disease but may reduce discomfort.

INDICATIONS FOR DELIVERY. Prolonged antepartum management of women with severe preeclampsia is not practiced in most centers. With improvements in neonatal care, many investigators regard delivery of women with rapidly progressing preeclampsia beyond 30 weeks' gestation to be in the best interest of not only the mother but also the fetus. When gestational age is critical (between 25 and 30 weeks), one might consider controlling maternal blood pressure along with meticulous observation of the maternal and fetal condition. Delivery is then indicated by worsening maternal symptoms, laboratory evidence of end-organ dysfunction, or deterioration of fetal condition. Whether this plan of action can effect a decrease in perinatal morbidity and mortality rates is not clear. The use of this approach with even very immature fetuses may only replace a nonviable neonate with an extremely premature one, with the attendant risk of long-range neurologic disability. Such an approach should therefore be attempted only in centers equipped to provide meticulous maternal observation and daily assessment of fetal and maternal condition.

FETAL INDICATIONS. The major consideration in decisions for delivery should usually be fetal well-being, for the reasons cited. Thus if the maternal condition is stable, delivery is indicated by signs of abnormal fetal function. If fetal growth and well-being remain normal, pregnancy should proceed to spontaneous labor. If the maternal condition is deteriorating rapidly, however, delivery is indicated for fetal well-being. With maternal deterioration, a reflection of increasingly poor perfusion of brain, kidney, and liver, uteroplacental blood flow is also likely to be compromised. In addition, the predictive value of all tests of fetal well-being is invalidated by rapid changes in the maternal and, hence, fetal condition.

MATERNAL INDICATIONS. Although fetal considerations usually dictate the timing of delivery, there are important exceptions. In the rare case in which a choice

is made to palliate maternal signs and symptoms to allow fetal growth or maturation, such efforts must be abandoned if the maternal condition worsens. Also, a potentially lethal complication of preeclampsia, hepatic rupture, cannot be prevented by any mode of therapy other than delivery. It has a mortality rate of 65%. Thus the woman with hepatic capsular distention manifested by hepatomegaly, tenderness of the liver, and abnormal hepatic function values should be delivered regardless of fetal well-being or maturity.

ROUTE OF DELIVERY. Vaginal delivery is preferable to cesarean delivery for women with preeclampsia. It is desirable, if possible, to avoid the added stress of surgery because of multiple physiologic abnormalities. Palliation for several hours should not increase maternal risk if performed appropriately. Induction should be carried out aggressively and expeditiously once the decision for delivery is made. In gestation remote from term in which delivery is indicated but fetal and maternal condition are stable enough to permit pregnancy to be prolonged 36 hours, glucocorticoids can be administered safely to accelerate fetal pulmonary maturity.

The aggressive approach to induction indicates that amniotomy be performed as soon as possible and a clear end point be formulated at the initiation of therapy, usually within 8 to 12 hours of the decision to induce delivery. A trial of induction is warranted regardless of cervical condition. Obviously, if vaginal delivery cannot be effected within the predetermined time frame, cesarean delivery should be considered. Likewise, cesarean delivery is performed for other usual obstetric indications.

A regional anesthetic such as epidural analgesia offers its usual advantages for vaginal and cesarean delivery *but does carry the possibility of extensive sympatholysis with consequent decreased cardiac output, hypotension, and impairment of already-compromised uteroplacental perfusion. This is a common problem with a spinal anesthetic, which is believed by most experts to be contraindicated in the woman with preeclampsia.* This problem can be avoided by meticulous attention to anesthetic technique and volume expansion. A regional anesthetic is not a rational means to lower blood pressure because it does so at the expense of cardiac output. Likewise, although analgesia with narcotics is not contraindicated and should be used when necesssary, there is abundant evidence that attempting to manage or prevent eclampsia with profound maternal sedation is dangerous and ineffective.

Drug treatment of hypertension related to preeclampsia. Disagreement exists on whether, and how efficiently, the uteroplacental blood flow is autoregulated. Those who liken the uterine circulation to a rigid conduit incapable of autoregulation caution against precipitous decrements in mean arterial pressure, because placental perfusion is already compromised in preeclampsia.[1, 2, 107] Others[108] prefer a more aggressive approach when treating hypertension. Resolution of this problem awaits perfection of reliable and safe methods to measure placental perfusion, followed by appropriately designed therapeutic trials. In the interim the following guidelines are recommended.

TREATMENT OF HYPERTENSION REMOTE FROM DELIVERY. As cited in several sections of this report, the palliative management of preeclampsia remote from delivery is controversial. The sole rationale is to allow maturation of the fetus and, if attempted, it must not subject the mother to undue risk. An important part of safe management is control of elevated blood pressure. In the woman with diastolic blood pressure 100 mm Hg or greater, risk is sufficient to warrant pharmacologic therapy. The therapy is solely for maternal benefit; there is neither theoretic basis nor empiric evidence that such therapy is beneficial to the fetus. If therapy is elected, methyldopa is the drug of choice. If this is not tolerated or is unsuccessful, calcium channel blockers, β-blockers, and hydralazine are reasonable additions or alternatives. Successful control of blood pressure should not be interpreted as eliminating risk for mother or infant. No evidence indicates that therapy improves fetal well-being or reduces the risk of abruptio placentae, disseminated intravascular coagulation, seizures, or other maternal risk. Both maternal and fetal assessment must be carried out meticulously regardless of the degree of blood pressure control.

TREATMENT OF ACUTE HYPERTENSION DURING DELIVERY. In the more usual situation, antihypertensive therapy occasionally may be indicated as acute palliative therapy in the woman in whom delivery is indicated. Antihypertensive agents can be withheld as long as maternal pressure is only mildly elevated. However, persistent diastolic levels of 105 mm Hg or higher should be treated.* When treatment is required, the ideal drug that reduces pressures to a safe level should:

- Act quickly
- Reduce pressure in a controlled manner
- Not lower cardiac output
- Reverse uteroplacental vascular constriction
- Result in no adverse maternal or fetal effects

The medications used to treat hypertensive crises in pregnancy are summarized in Table II. Details of their pharmacology and safety are discussed elsewhere.[85, 107, 109]

The current drug of first choice is intravenous hydralazine, which if given cautiously is successful in most instances. It has been shown to be effective against preeclamptic hypertension.[34] Although this is sometimes

*It may, however, be prudent to treat lower levels in certain situations (e.g., the young gravid woman whose recent diastolic levels were below 75 mm Hg or the woman with chronic hypertension for many years and in whom hypertensive heart disease may be present).

Table II. Guidelines for drug treatment of severe hypertension near term or during labor*

- Hydralazine administered intravenously is the drug of choice. Use low doses (start with a 5 mg intravenous bolus and then give 5 to 10 mg every 20 to 30 minutes to avoid precipitous decreases. Side effects include tachycardia and headache. Neonatal thrombocytopenia has been reported.
- Diazoxide is recommended for the occasional patient whose hypertension is refractory to hydralazine. Use 30 mg miniboluses, because maternal vascular collapse and death have been associated with the customary 300 mg dose. Side effects include arrest of labor and neonatal hyperglycemia.
- Experience with parenteral labetalol is growing, and this drug may replace diazoxide as the second-line drug.
- Favorable results have been reported with calcium channel blockers. However, if magnesium sulfate is being infused, the magnesium ion may potentiate the effect of calcium channel blockers, resulting in precipitous and severe hypotension.
- Do not use sodium nitroprusside (fetal cyanide poisoning has been reported in animal models) or diuretics (e.g., furosemide; see text). However, in the final analysis, maternal well-being will dictate the choice of therapy.

*The degree to which blood pressure should be decreased is disputed. Levels from 90 to 104 mm Hg diastolic are recommended (see text).

used as an intravenous infusion, the pharmacokinetics (maximal effect at 20 minutes; duration of action 6 to 8 hours) indicate that intermittent bolus injections are more sensible. A 5 mg bolus is given intravenously for 1 to 2 minutes. Twenty minutes later, subsequent doses are dictated by the initial response. Once the desired effect is obtained, the drug is repeated as necessary (frequently in several hours). If a total of 20 mg is administered without therapeutic response, other agents should be considered.

Diazoxide is restricted to the occasional resistant case and should be administered in small doses (30 mg boluses). Preliminary successes have been recorded when calcium channel blockers (e.g., nifedipine) have been used, and in 1989 this group of drugs was undergoing testing.[107, 110] One concern about calcium channel blockers, however, is that most patients with acute hypertension during delivery will also be receiving magnesium sulfate (see below). Magnesium may potentiate the effects of calcium channel blockers and lead to precipitous decreases in blood pressure. Nifedipine acts rapidly, causing significant reduction in arterial blood pressure within 10 to 20 minutes of oral administration. The onset of antihypertensive activity can possibly be shortened by chewing the capsule or puncturing it with several needle holes before it is swallowed. The principal side effects are headache and cutaneous flushing, but minimal reflex tachycardia may occur. Like vasodilators, calcium channel blockers may cause cessation of uterine contractions. They are used to stop premature labor without maternal or fetal side effects.

Limited data are available on the use of parenteral labetalol (which some advocate as a second-line drug), starting with 10 mg and not to exceed 1 mg/kg, or clonidine. Neither labetalol nor clonidine appears to be more effective than hydralazine. Sodium nitroprusside is chosen only after the failure of hydralazine, diazoxide, calcium channel blockers, labetalol, and clonidine because of cyanide poisoning and fetal death reported in laboratory animals.[111]

Finally, the use of potent saluretic agents such as furosemide in treating hypertensive crises at term, as adjunct therapy to the vasodilators just discussed, is condemned by most authorities but still has its advocates.[108] Given the hemoconcentration and cardiovascular hemodynamics of preeclampsia and the susceptibility of some women with this disease to either intraportal hypotension or puerperal vascular collapse, many counsel against the use of potent loop diuretics and care must be taken with all antihypertensive agents. In the last analysis, however, the mother's well-being should take precedence, even if the therapy necessary to control pressure may potentially harm the fetus.

ANTIECLAMPTIC THERAPY. The pathophysiology of eclampsia is discussed in the section on Pathology and Pathophysiology of Hypertension in Pregnancy Focusing on Preeclampsia. It is a mistake to equate the eclamptic convulsion with hypertensive encephalopathy, because *the convulsion* can arise in a seemingly stable patient manifesting only minimal elevation in blood pressure. For this reason many clinicians initiate prophylactic therapy when women with suspected preeclampsia are in labor, even if premonitory signs are absent.

Because the pathogenesis of the eclamptic convulsion is still poorly understood,[107] it is not surprising to find disagreements on how to treat women with impending convulsions or frank eclampsia.[2, 107, 112-114] Most authorities, especially in North America, use parenterally administered magnesium sulfate[34]; others prefer conventional anticonvulsant drugs such as diazepam and phenytoin. Critics of the use of magnesium sulfate stress that it crosses the blood-brain barrier very slowly[115] and has no effect on electroencephalographic abnormalities.[116] Defense of magnesium sulfate therapy has been mainly empiric, but of interest are recent observations of the effect of magnesium ions on prostaglandin metabolism. For example, Watson et al.[117] have demonstrated that magnesium at levels measured in treated patients with preeclampsia increases prostacyclin release by cultured endothelial cells from human umbil-

ical veins and plasma from women with preeclampsia treated with magnesium sulfate had similar actions. The preference for magnesium sulfate, especially in the United States, is documented by its successful use in several large series,[1, 31, 108, 117, 118] but it has never undergone a definitive controlled trial. Similarly, there is a need for more extensive data regarding the effect of both magnesium and standard anticonvulsant drugs on the neonate. Preliminary data on the effects of magnesium sulfate on fetuses are encouraging.[119]

VOLUME EXPANSION THERAPY. Just as there are advocates of saluretic agents, there are claims that volume expansion may reduce blood pressure in selected patients with preeclampsia. This approach derives from observations that plasma volume, cardiac output, and pulmonary capillary wedge pressure may be decreased in this condition, as well as from reports that infusion of colloids decreased blood pressure[33, 120] and peripheral vascular resistance,[33] despite increments in intravascular volume. However, the effects of colloid infusion are usually transient, probably because the vasculature in preeclampsia is "leaky,"[121] whereas infusion of crystalloids alone decreases oncotic pressure, which is already depressed in preeclampsia.[122] Such decrements can lead to pulmonary or cerebral edema, especially in the immediate puerperium, when oncotic pressure levels decrease further, whereas central volume and pulmonary capillary wedge pressure tend to rise.[31, 122] Thus one should be cautious concerning crystalloid infusions into women with preeclampsia during labor and until a postpartum diuresis is established. Signs suggesting poor renal perfusion (i.e., oliguria) resolve quickly after delivery, and acute renal failure is an unusual complication, even in severe preeclampsia.

OTHER CONSIDERATIONS. Invasive cardiovascular monitoring may be required in severe or complicated cases, especially during operative procedures. Criteria have recently been proposed for pulmonary artery catheterization in the patient with severe preeclampsia.[123] Many experts, however, believe that these criteria are too broad and find the indications for use of Swan-Ganz catheters (a procedure associated with a certain morbidity) relatively uncommon.[124]

Treating hypertension persisting post partum. The potential problems of serious compromise of placental perfusion and, in turn, fetal well-being that can be induced by antihypertensive agents are obviated by delivery. If there is a problem after delivery controlling persisting severe hypertension, intermittent intravenous hydralazine can be used repeatedly early in the puerperium to control it. Once repeated blood pressure readings remain near normal, the hydralazine is stopped and treatment with standard oral regimens should be started.

Several other regimens are effective for control of severe postpartum hypertension. These have been discussed in the Joint National Committee Report[7] and include infusion of nitroprusside (0.5 to 10 mg/kg/min). Labetalol, 20 to 80 mg by intravenous bolus, lowers blood pressure in 5 to 10 minutes and may be repeated at 10-minute intervals.

Acute hypertensive changes induced by pregnancy usually dissipate rapidly after delivery, certainly within the first several days. If severe hypertension persists more than 3 to 5 days, the likelihood of underlying chronic hypertension is greatly increased. In these cases oral antihypertensive therapy is begun before discharge and the woman is evaluated in 1 week. For women who were hypertensive before pregnancy, chronic treatment is likely to be necessary. If prepregnancy blood pressure was normal or unknown, it is reasonable to stop oral medication after 3 to 4 weeks and observe the blood pressure at weekly intervals for 1 month and at monthly intervals for 1 year. If hypertension recurs, treatment should be resumed.

Lactation. Many women who have been chronically hypertensive during pregnancy will have the desire to breast-feed their infants for a period of several weeks to 1 year. The concentrations of most of the antihypertensive drugs have been assessed in human breast milk and plasma[125] after single or multiple dosings and all agents studied have been detectable in the milk. However, only a few reports have evaluated whether the drug is detectable in the plasma of the breast-fed infant[126-128] or if there is any hemodynamic or adverse effect of the agent on the infant. Furthermore, there have been no clinical trials involving several subjects that have studied the cardiovascular effects of any antihypertensive agent on the breast-fed infant.

In mothers with mild hypertension who wish to breast-feed for a few months, the clinician may consider withholding medication with close observation of the maternal blood pressure. After discontinuation of the nursing period, the antihypertensive therapy should be reinstituted as appropriate. For those patients with more severe elevation of blood pressure taking a single antihypertensive agent, the clinician may consider reducing antihypertensive drug dosage with close observation of both the mother and breast-fed infant. If the mother requires multiple agents for controlling the hypertension, breast-feeding is not advisable.

Remote prognosis

Eclampsia. The weighted average prevalence of hypertension after eclampsia is 23.8%, as derived from 53 articles reporting follow-up studies of 2637 women.[1] There are only two studies of large series with long-term follow-up, and they agree that the prevalence of remote hypertension is not increased over that in unselected women matched for age and race. Bryans[129] at

the Medical College of Georgia followed up 335 women for 1 to 44 years (average 14). The average age-specific blood pressures of 168 white women at follow-up fell in the middle of averages for unselected women in three epidemiologic studies of blood pressure, and there was no excess of women with diastolic pressures of 90 mm Hg or higher. The average age-specific blood pressures of 167 black women were higher than in white women but were within the range of such pressures in unselected black women.

For an average of 33 years, Chesley et al.[130] followed up 99% of the 270 women who survived eclampsia between 1931 and 1951 in the Margaret Hague Maternity Hospital in New Jersey, with all but 3 traced to 1974. Remote deaths among the 206 women who had eclampsia when primiparous did not exceed the number expected, as derived from age-specific death rates for American women matched for age and race. Only 29% of the remote deaths were associated with hypertensive vascular disease. In contrast, three times the expected number of deaths occurred among the 64 women who had had eclampsia when multiparous, and 80% of the deaths were caused by cardiovascular-renal complications. Chronic hypertension had been known to antedate the eclampsia in several of the women. The lowest expected prevalence of hypertension among 11 epidemiologic studies of blood pressure was reported in the study by Hamilton et al.[131]; therefore their study was used as the most rigid control. The distributions of diastolic and systolic pressures at an average of 33 years after eclampsia in primiparous women were virtually identical to those of the control subjects matched for age, and there was no excess of patients with hypertension among the women with posteclampsia, whatever blood pressure might be taken as the dividing line. There was an excess of cases of hypertension among the multiparous women.

Preeclampsia. Many of the numerous articles describing follow-up studies of women thought to have had preeclampsia have found high prevalences of hypertension, and some authors have believed that preeclampsia may cause chronic hypertension in women who otherwise never would have had it. It is significant that such "residual" hypertension is more common after multiparous than after primiparous pregnancies, more common after mild than severe preeclampsia, and lowest of all after eclampsia. In a word, the more secure the diagnosis, the lower the rate of ultimate hypertension. In the past, and still in most classifications, the diagnosis of mild preeclampsia is made in women with transient hypertension (see the following section). In the unique study by Fisher et al.[19] the diagnosis of preeclampsia was confirmed by renal biopsy. The prevalence of hypertension at follow-up was about 10%, almost identical to the expected rate as derived from women in the National Health Survey when matched for age and race.

Thus the prevalence of chronic hypertension after eclampsia[129, 130] or preeclampsia[13] in primigravid women is the same as in unselected women matched for age and race. Preeclampsia-eclampsia is an intercurrent event, unrelated to chronic hypertension (except that chronic hypertension may predispose to it).

Transient hypertension. Also called gestational or late hypertension, transient hypertension is the diagnosis for mere rises in blood pressure without significant edema or proteinuria, with a return of the blood pressure to normal within 10 days after delivery. Follow-up studies by Berman,[16] Herrick and Tillman,[15] Chesley et al.,[130] Adams and MacGillivray,[17] and others indicate that transient hypertension usually recurs in later pregnancies and often predicts ultimate chronic hypertension. It probably most often is latent essential hypertension unmasked by pregnancy.

REFERENCES

1. Chesley LC. Hypertensive disorders in pregnancy. New York: Appleton-Century-Crofts, 1978.
2. Cunningham FG, Leveno KJ. Management of pregnancy-induced hypertension. In: Rubin PC, ed. Handbook of hypertension, vol 10. Hypertension in pregnancy. Amsterdam: Elsevier, 1988:290-319.
3. Pollak VE. Pre-eclampsia and kidney disease. In: Coggins CH, Cummings NB, eds. Prevention of kidney and urinary tract diseases. DHEW Publication No. (NIH) 78-855, 1978:95-129.
4. Robinson N. Salt in pregnancy. Lancet 1958;1:178-81.
5. MacGillivray I. Some observations on the incidence of preeclampsia. J Obstet Gynaecol Br Empire 1958;65: 536-9.
6. Thompson AM, Chun D, Baird D. Perinatal mortality in Hong Kong and in Aberdeen, Scotland. J Obstet Gynaecol Br Commonwealth 1963;70:871-7.
7. The 1988 Joint National Committee. The 1988 report of the Joint Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med 1988;148:1023-38.
8. Hughes EC, ed. Obstetric-gynecologic terminology. Philadelphia: Davis, 1972:422-3.
9. American College of Obstetricians and Gynecologists. Management of preeclampsia. ACOG Technical Bulletin Feburary 1986, No. 91.
10. World Health Organization Study Group. The hypertensive disorders of pregnancy. WHO Technical Report Series No. 758. Geneva: World Health Organization, 1987.
11. Davey DA, MacGillivray I. The classification and definition of the hypertensive disorders of pregnancy. Clin Exp Hypertens Pregnancy 1986;B5:97-133.
12. Chesley LC, Annitto JE. Pregnancy in the patient with hypertensive disease. AM J OBSTET GYNECOL 1947;53: 372-81.
13. Dieckmann WJ. The toxemias of pregnancy. 2nd ed. St. Louis: CV Mosby, 1952:422.
14. Sibai BM, McCubbin JH, Anderson GD, Lipshitz J, Dilts PV Jr. Eclampsia. I. Observations from sixty-seven recent cases. Obstet Gynecol 1981;58:609-13.
15. Herrick WW, Tillman AJB. The mild toxemias of late pregnancy: their relation to cardiovascular and renal disease. AM J OBSTET GYNECOL 1936;31:832-44.
16. Berman S. Observations in the toxemia clinic, Boston Lying-in Hospital, 1923-1930. N Engl J Med 1930;203: 361-3.
17. Adams EM, MacGillivray I. Long-term effect of preeclampsia on blood pressure. Lancet 1961;2:1373-5.
18. Redman CW, Beilin LJ, Bonnar J, et al. Plasma urate

measurements in predicting fetal death in hypertensive pregnancy. Lancet 1976;1:1370-3.
19. Fisher KA, Luger A, Spargo BH, Lindheimer MD. Hypertension in pregnancy: clinical-pathological correlations and remote prognosis. Medicine 1981;60:267-76.
20. Aarnoudse JG, Houthoff HJ, Weits J, Vellenga E, Huisjes HJ. A syndrome of liver damage and intravascular coagulation in the last trimester of normotensive pregnancy: a clinical and histopathological study. Br J Obstet Gynaecol 1986;93:145-55.
21. Hinselmann H. Allgemeine Krankheitslehre. In: Hinselmann H, ed. Die Eklampsie. Bonn: Cohen, 1924:1-87.
22. Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. Br J Obstet Gynaecol 1986;93:898-908.
23. White P. Pregnancy complicating diabetes. Surg Gynecol Obstet 1935;61:324-32.
24. Page EW. The relation between hydatid moles, relative ischemia of the gravid uterus, and the placental origin of eclampsia. AM J OBSTET GYNECOL 1939;37:291-3.
25. Jann R, Spätgestosen bei Hydrops fetus et placentae infolge Rhesus-Inkompatibilität. Arch Gynaekol 1954; 184:731-48.
26. Scott JS. Pregnancy toxaemia associated with hydrops foetalis, hydatidiform mole, and hydramnios. J Obstet Gynaecol Br Empire 1958;65:689-701.
27. Nelson TR. A clinical study of pre-eclampsia, pts I and II. J Obstet Gynaecol Br Empire 1955;62:48-66.
28. Redman CW. Eclampsia still kills. Br Med J 1988;296: 1209-10.
29. Talledo OE, Chesley LC, Zuspan FP. Renin-angiotensin system in normal and toxemic pregnancies. III. Differential sensitivity to angiotensin II and norepinephrine in toxemia of pregnancy. AM J OBSTET GYNECOL 1968; 100:218-21.
30. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest 1973; 52:2682-9.
31. Hankins GD, Wendel GD Jr, Cunningham FG, Leveno KJ. Longitudinal evaluation of hemodynamic changes in eclampsia. AM J OBSTET GYNECOL 1984;150:506-12.
32. Groenendijk R, Trimbros JB, Wallenburg HC. Hemodynamic measurements in preeclampsia: preliminary observations. AM J OBSTET GYNECOL 1984;150: 232-6.
33. Wallenburg HCS. Hemodynamics in hypertensive pregnancy. In: Ruben PC, ed. Handbook of hypertension, vol 10. Hypertension in pregnancy. Amsterdam: Elsevier, 1988:66-101.
34. Pritchard JA, Cunningham FG, Pritchard SA. The Parkland Memorial Hospital protocol for treatment of eclampsia: evaluation of 245 cases. AM J OBSTET GYNECOL 1984;148:951-63.
35. Pritchard JA, Cunningham FG, Mason RA. Coagulation changes in eclampsia: their frequency and pathogenesis. AM J OBSTET GYNECOL 1976;124:855-9.
36. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. AM J OBSTET GYNECOL 1989;161:1200-4.
37. Saleh AA, Bottoms SF, Welch RA, Ali AM, Mariona FG, Mammen EF. Preeclampsia, delivery and the hemostatic system. AM J OBSTET GYNECOL 1987;157:331-6.
38. Burrows RF, Hunter DJ, Andrew M, Kelton JG. A prospective study investigating the mechanism of thrombocytopenia in preeclampsia. Obstet Gynecol 1987;70: 334-8.
39. Pritchard JA, Cunningham FG, Pritchard SA, Mason RA. How often does maternal preeclampsia-eclampsia incite thrombocytopenia in the fetus? Obstet Gynecol 1987;69:292-5.
40. Gaber LW, Spargo BH, Lindheimer MD. Renal pathology in preeclampsia. Clin Obstet Gynaecol (Bailliere) 1987;1:971-95.
41. Chesley LC. Diagnosis of preeclampsia. Obstet Gynecol 1985;65:423-5.
42. Spargo B, McCartney CP, Winemiller R. Glomerular capillary endotheliosis in toxemia of pregnancy. Arch Pathol 1959;68:593-9.
43. Sibai BM, Taslimi MM, el Nazer A, Amon E, Mabie BC, Ryan GM. Maternal-perinatal outcome associated with the syndrome of hemolysis, elevated liver enzymes and low platelets in severe preeclampsia-eclampsia. AM J OBSTET GYNECOL 1986;155:501-9.
44. Sheehan HL. Pathologic lesions in the hypertensive toxemias of pregnancy. In: Hammond J, Browne FJ, Wolstenholm GEW, eds. Toxemias of pregnancy: human and veterinary. Philadelphia: Blakiston, 1950:16-22.
45. McCall ML. Cerebral circulation and metabolism in toxemia of pregnancy: observations on the effects of Veratrum viride and Apresoline (1-hydrazinophthalazine). AM J OBSTET GYNECOL 1953;66:1015-30.
46. Sibai BM, Spinnato JA, Watson DL, Lewis JA, Anderson GD. Eclampsia. IV. Neurological findings and future outcome. AM J OBSTET GYNECOL 1985;152:184-92.
47. Brown CE, Purdy P, Cunningham FG. Head computed tomographic scans in women with eclampsia. AM J OBSTET GYNECOL 1988;159:915-20.
48. Sheehan HL, Lynch JB, eds. Cerebral lesions. In: Pathology of toxaemia of pregnancy. Baltimore: Williams & Wilkins, 1973:524-53.
49. Browne JCM, Veall N. The maternal placental blood flow in normotensive and hypertensive women. J Obstet Gynaecol Br Empire 1953;60:141-7.
50. Fleischer A, Schulman H, Farmakides G, et al. Uterine artery Doppler velocimetry in pregnant women with hypertension. AM J OBSTET GYNECOL 1986;154:806-13.
51. Trudinger BJ, Giles WB, Cook CM. Flow velocity waveforms in the maternal uteroplacental and fetal umbilical placental circulations. AM J OBSTET GYNECOL 1985;152: 155-63.
52. Sibai BM, Taslimi M, Abdella TN, Brooks TF, Spinnato JA, Anderson GD. Maternal and perinatal outcome of conservative management of severe preeclampsia in midtrimester. AM J OBSTET GYNECOL 1985; 152:32-7.
53. Weiner CP, Kwaan HC, Xu C, Paul M, Burmeister L, Hauck W. Antithrombin III activity in women with hypertension during pregnancy. Obstet Gynecol 1985;65: 301-6.
54. Entman SS, Kambam JR, Bradley CA, Cousar JB. Increased levels of carboxyhemoglobin and serum iron as an indicator of increased red cell turnover in preeclampsia. AM J OBSTET GYNECOL 1987;156:1169-73.
55. Taufield PA, Ales KL, Resnick LM, Druzin ML, Gertner JM, Laragh JH. Hypocalciuria in preeclampsia. N Engl J Med 1987;316:715-8.
56. Lindheimer MD, Katz AI. Preeclampsia: pathophysiology, diagnosis, and management. Annu Rev Med 1989; 40:233-50.
57. Foidart JM, Hunt J, Lapiere CM, et al. Antibodies to laminin in preeclampsia. Kidney Int 1986;29:1050-7.
58. Rodgers GM, Taylor RN, Roberts JM. Preeclampsia is associated with a serum factor cytotoxic to human endothelial cells. AM J OBSTET GYNECOL 1988;159: 908-14.
59. Musci TJ, Roberts JM, Rodgers GM, Taylor RN. Mitogenic activity is increased in the sera of preeclamptic women before delivery. AM J OBSTET GYNECOL 1988; 159:1446-51.
60. Ellison GT, Mansberger JA, Mansberger AR Jr. Malignant recurrent pheochromocytoma during pregnancy: case report and review of the literature. Surgery 1988; 103:484-9.
61. Schenker JG, Chowers I. Pheochromocytoma and pregnancy: review of eighty-nine cases. Obstet Gynecol Surv 1971;26:739-47.
62. Packham DK, Fairley KF, Ihle BU, Whitworth JA, Kincaid-Smith P. Comparison of pregnancy outcome between normotensive and hypertensive women with pri-

mary glomerulonephritis. Clin Exp Hypertens [A] 1987-1988;B6:387-99.
63. Hou SH, Grossman SD, Madias NE. Pregnancy in women with renal disease and moderate renal insufficiency. Am J Med 1985;78:185-94.
64. Lindheimer MD, Katz AI. Gestation in women with kidney disease: prognosis and management. Clin Obstet Gynaecol (Bailliere) 1987;1:921-37.
65. Papiernik E, Kaminski M. Multifactorial study of the risk of prematurity at thirty-two weeks of gestation. I: A study of the frequency of thirty predictive characteristics. J Perinat Med 1974;2:30-5.
66. Gallery ED, Hunyor SN, Györy AZ. Plasma volume contraction: a significant factor in both pregnancy-associated hypertension (pre-eclampsia) and chronic hypertension in pregnancy. Q J Med 1979;48:593-602.
67. Palomaki JF, Lindheimer MD. Sodium depletion simulating deterioration in a toxemic pregnancy. N Engl J Med 1970;282:88-9.
68. Kawasaki N, Matsui K, Ito M, et al. Effect of calcium supplementation on the vascular sensitivity to angiotensin II in pregnant women. AM J OBSTET GYNECOL 1985;153:576-82.
69. Villar J, Repke J, Belizan JM, Pareja G. Calcium supplementation reduces blood pressure during pregnancy: results of a randomized controlled clinical trial. Obstet Gynecol 1987;70:317-22.
70. Rayburn WF, Zuspan FP, Piehl EJ. Self-monitoring of blood pressuring during pregnancy. AM J OBSTET GYNECOL 1984;148:159-62.
71. Page EW, Christianson R. The impact of mean arterial blood pressure in the middle trimester upon the outcome of pregnancy. AM J OBSTET GYNECOL 1976;125:740-5.
72. Friedman EA, Neff RK. Pregnancy hypertension: a systematic evaluation of clinical diagnostic criteria. Littleton, MA: PSG, 1977.
73. Dunlop JCH. Chronic hypertension and perinatal mortality. Proc R Soc Med 1966;59:838-41.
74. Leather HM, Humphreys DM, Baker P, Chadd MA. A controlled trial of hypotensive agents in hypertension in pregnancy. Lancet 1968;2:488-90.
75. Rubin PC, Butters L, Clark DM, et al. Placebo-controlled trial of atenolol in treatment of pregnancy-associated hypertension. Lancet 1983;1:431-4.
76. Wichman K, Ryden G, Karlberg BE. A placebo-controlled trial of metoprolol in the treatment of hypertension in pregnancy. Scand J Clin Lab Invest Suppl 1984;169:90-5.
77. Landesman R, McLarn WD, Ollstein RN, Mendelsohn B. Reserpine in toxemia of pregnancy. Obstet Gynecol 1957;9:377-83.
78. Fletcher AE, Bulpitt CJ. A review of clinical trials in pregnancy. In: Rubin PC, ed. Hypertension in pregnancy. New York: Elsevier, 1988:186-201.
79. Redman CW, Beilin LJ, Bonnar J, Ounsted MK. Fetal outcome in trial of antihypertensive treatment in pregnancy. Lancet 1976;2:753-6.
80. Ferris TF, Weir EK. Effect of captopril on uterine blood flow and prostaglandin E synthesis in the pregnant rabbit. J Clin Invest 1983;71:809-15.
81. Schubiger G, Flury G, Nussberger J. Enalapril for pregnancy-induced hypertension: acute renal failure in a neonate. Ann Intern Med 1988;108:215-6. (Published erratum appears in Ann Intern Med 1988;108:777.)
82. Rosa FW, Bosco LA, Graham CF, Milstien JB, Dreis M, Creamer J. Neonatal anuria with maternal angiotensin-converting enzyme inhibition. Obstet Gynecol 1989;74:371-4.
83. Scott AA, Purohit DM. Neonatal renal failure: a complication of maternal antihypertensive therapy. AM J OBSTET GYNECOL 1989;160:1223-4.
84. Knott PD, Thorpe SS, Lamont CAR. Congenital renal dysgenesis possibly due to captopril. Lancet 1989;1:451.
85. Barron WM, Murphy MD, Lindheimer MD. Management of hypertension during pregnancy. In: Laragh JH, Brenner BM, eds. Hypertension, diagnosis and management. New York: Raven Press, 1990:1809-27.
86. Redman CW. A controlled trial of the treatment of hypertension in pregnancy: labetalol compared with methyldopa. In: Riley A, Symonds EM, eds. Investigation of labetalol in the management of hypertension in pregnancy. International Congress series 591. Amsterdam: Excerpta Medica, 1982:101-10.
87. Sibai BM, Gonzalez AR, Mabie WC, Moretti M. A comparison of labetalol plus hospitalization versus hospitalization alone in the management of preeclampsia remote from term. Obstet Gynecol 1987;70:323-7.
88. Sibai BM, Mabie WC, Villar M, Shamsa F, Anderson GD. A comparison of no medication versus methyldopa or labetalol in chronic hypertension during pregnancy. AM J OBSTET GYNECOL 1990;162:960-6.
89. Butters L, Kennedy S, Rubin P. Atenolol and fetal weight in chronic hypertension [Abstract]. Clin Exp Hypertens [A] 1989;B8:468.
90. Constantine G, Beevers DG, Reynolds AL, Luesley DM. Nifedipine as a second-line antihypertensive drug in pregnancy. Br J Obstet Gynaecol 1987;94:1136-42.
91. Ulmsten U. Treatment of normotensive and hypertensive patients with preterm labor using oral nifedipine, a calcium antagonist. Arch Gynecol 1984;236:69-72.
92. Fidler J, Smith V, Fayers P, DeSwiet M. Randomized controlled comparative study of methyldopa and oxprenolol in treatment of hypertension in pregnancy. Br Med J 1983;286:1927-30.
93. Kincaid-Smith P, Bullen M, Mills J. Prolonged use of methyldopa in severe hypertension in pregnancy. Br Med J 1966;1:274-6.
94. Redman CW. Treatment of hypertension in pregnancy. Kidney Int 1980;18:267-78.
95. Ounsted M, Cockburn J, Moar VA, Redman CW. Maternal hypertension with superimposed pre-eclampsia: effects of child development at 7½ years. Br J Obstet Gynaecol 1983;90:644-9.
96. Hays PM, Cruikshank DP, Dunn LJ. Plasma volume determination in normal and preeclamptic pregnancies. AM J OBSTET GYNECOL 1985;151:958-66.
97. Arias F, Zamora J. Antihypertensive treatment and pregnancy outcome in patients with mild chronic hypertension. Obstet Gynecol 1979;53:489.
98. Sibai BM, Grossman RA, Grossman HG. Effects of diuretics on plasma volume in pregnancies with long-term hypertension. AM J OBSTET GYNECOL 1984;150:831-5.
99. Kraus GW, Marchese JR, Yen SSC. Prophylactic use of hydrochlorothiazide in pregnancy. JAMA 1966;198:1150-4.
100. Collins R, Yusuf S, Peto R. Overview of randomised trials of diuretics in pregnancy. Br Med J 1985;290:17-23.
101. Widerlov E, Karlman I, Storsater J. Hydralazine-induced neonatal thrombocytopenia (letter). N Engl J Med 1980;301:1235.
102. Beaufils M, Uzan S, Donsimoni R, Colau JC. Prevention of pre-eclampsia by early antiplatelet therapy. Lancet 1985;1:840-2.
103. Wallenburg HC, Dekker GA, Makovitz JW, Rotmans P. Low-dose aspirin prevents pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae. Lancet 1986;1:1-3.
104. Schiff E, Peleg E, Goldenberg M, et al. The use of aspirin to prevent pregnancy-induced hypertension and lower the ratio of thromboxane A_2 to prostacyclin in relatively high-risk pregnancies. N Engl J Med 1989;321:351-6.
105. Benigni A, Gregorini G, Frusca T, et al. Effect of low-dose aspirin on fetal and maternal generation of thromboxane by platelets in women at risk for pregnancy-induced hypertension. N Engl J Med 1989;321:357-62.

106. Stuart MJ, Gross SJ, Elrad H, Graeber JE. Effects of acetylsalicylic-acid ingestion on maternal and neonatal hemostasis. N Engl J Med 1982;307:909-12.
107. Davison JM, Lindheimer MD. Hypertension and pregnancy. In: Schrier RW, Gottschalk CW, eds. Diseases of the kidney, vol 2. 4th ed. Boston: Little Brown & Co, 1988:1653-86.
108. Ferris TF. How should hypertension during pregnancy be managed? An internist's approach. Med Clin North Am 1984;68:491-503.
109. Naden RP, Redman CW. Antihypertensive drugs in pregnancy. Clin Perinatol 1985;12:521-38.
110. Walters BN, Redman CW. Treatment of severe pregnancy-associated hypertension with the calcium antagonist nifedipine. Br J Obstet Gynaecol 1984;91: 330-6.
111. Naulty J, Cefalo RC, Lewis PE. Fetal toxicity of nitroprusside in the pregnant ewe. AM J OBSTET GYNECOL 1981;139:708-11.
112. Ferris TF. Prostanoids in normal and hypertensive pregnancy. In: Rubin PC, ed. Hypertension in pregnancy. New York: Elsevier, 1988:102-17.
113. Dinsdale HB. Does magnesium sulfate treat eclamptic seizures? Yes. Arch Neurol 1988;45:1360-1.
114. Kaplan PW, Lesser RP, Fisher RS, Repke JT, Hanley DF. No, magnesium sulfate should not be used in treating eclamptic seizures. Arch Neurol 1988;45:1361-4.
115. Thurnau GR, Kemp DB, Jarvis A. Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: a preliminary report. AM J OBSTET GYNECOL 1987;157:1435-8.
116. Sibai BM, Spinnato JA, Watson DL, Lewis JA, Anderson GD. Effect of magnesium sulfate on electroencephalographic findings in preeclampsia-eclampsia. Obstet Gynecol 1984;64:261-6.
117. Watson KV, Moldow CF, Ogburn PL, Jacob HS. Magnesium sulfate: rationale for it use in preeclampsia. Proc Natl Acad Sci USA 1986;83:1075-8.
118. Sibai BM, Anderson GD. Pregnancy outcome of intensive therapy in severe hypertension in first trimester. Obstet Gynecol 1986;67:517-22.
119. Pruett KM, Kirshon B, Cotton DB, Adam K, Doody KJ. The effects of magnesium sulfate therapy on Apgar scores. AM J OBSTET GYNECOL 1988;159:1047-8.
120. Gallery ED, Delprado W, Györy AZ. Antihypertensive effect of plasma volume expansion in pregnancy-associated hypertension. Aust NZ J Med 1981;11:20-4.
121. Øian P, Maltau JM, Noddeland H, Fadnes HO. Transcapillary fluid balance in pre-eclampsia. Br J Obstet Gynaecol 1986;93:235-9.
122. Zinaman M, Rubin J, Lindheimer MD. Serial plasma oncotic pressure levels and echoencephalography during and after delivery in severe pre-eclampsia. Lancet 1985;1:1245-7.
123. Clark SL, Cotton DB. Clinical indications for pulmonary artery catheterization in the patient with severe preeclampsia. AM J OBSTET GYNECOL 1988;158:453-8.
124. American College of Obstetricians and Gynecologists. Invasive hemodynamic monitoring in obstetrics and gynecology. ACOG Technical Bulletin October 1988, No. 121.
125. White WB. Management of hypertension during lactation. Hypertension 1984;6:297-300.
126. White WB, Andreoli JW, Cohn RD. Alpha-methyldopa disposition in mothers with hypertension and in their breast-fed infants. Clin Pharmacol Ther 1985;37:387-90.
127. Miller ME, Cohn RD, Burghart PH. Hydrochlorothiazide disposition in a mother and her breast-fed infant. J Pediatr 1982;101:789-91.
128. Krause W, Stoppelli I, Milia S, Rainer E. Transfer of mepindolol to newborns by breast-feeding mothers after single and repeated daily doses. Eur J Clin Pharmacol 1982;22:53-5.
129. Bryans CI Jr. The remote prognosis in toxemia of pregnancy. Clin Obstet Gynecol 1966;9:973-90.
130. Chesley LC, Annitto JE, Cosgrove RA. The remote prognosis of eclamptic women: sixth periodic report. AM J OBSTET GYNECOL 1976;124:446-59.
131. Hamilton M, Pickering GW, Roberts JAF, Sowry GSC. The aetiology of essential hypertension: the arterial blood pressure in the general population. Clin Sci 1954; 13:11-35.
132. Frohlich ED, Grim C, Labarthe DR, Maxwell MH, Perloff D, Weidman W. Recommendations for human blood pressure determination by sphygmomanometers: report of a special task force appointed by the Steering Committee, American Heart Association. Hypertension 1988;11:209A-22A.

Appendix

Blood pressure measurement protocol. Accurate and consistent blood pressure measurements are essential to establish a baseline and monitor subtle changes throughout the duration of the pregnancy. Blood pressures should be taken in a standardized fashion by all personnel who see the pregnant woman during her antepartum, intrapartum, and postpartum care. In 1987 the American Heart Association (AHA) updated its standards for the fifth time since 1939 in its Recommendations for Human Blood Pressure Determination by Sphygmomanometers.[132] Although this document focuses primarily on nonpregnant populations, many of its paradigms relate to the gestational period as well.

For accurate blood pressure measurements in all clinical situations, the AHA document recommends a standardized measurement technique. This includes:

- Have the patient sit* for 5 minutes before blood pressure measurement with the arm resting on a table at heart level (using the same arm on each measurement occasion for consistency).
- Measure the bared arm for proper cuff size and have available commonly used cuff sizes for adults, notably regular and large adult cuffs with respective bladder widths of 12 and 15 cm (or in general, a bladder width of 40% of the arm circumference, ensuring a bladder length that encircles 80% of the upper arm).
- Assess for approximate systolic blood pressure level before measurement by finding where the radial pulse disappears with simultaneous palpation and inflation to disappearance.
- Maintain a slow, steady deflation rate of 2 to 3 mm Hg per second or per heartbeat when doing a reading.
- Take the average of the two readings (also recommended by the Joint National Committee) to help minimize variations in recorded blood pressure across time caused by blood pres-

*The WHO Study Group[10] notes that it probably does not matter whether the patient sits or lies slightly on the left side (15- to 30-degree tilt), provided the same position is used. For practical purposes the seated position with the arm resting at heart level is the procedure used in virtually all prenatal clinics.

sure's inherent variability (reflected in isolated readings).
- Use accurate equipment (mercury sphygmomanometer or calibrated aneroid).

It is important to remember that blood pressure measurements are influenced by the relationship of the cuff to the level of the heart (caused by hydrostatic pressure, the effect of gravity on the column of blood). Blood pressure with the cuff higher than the level of the heart will be lower; blood pressure with the cuff lower than the level of the heart will be higher. For example, the practice of taking the blood pressure in the upper arm with the woman on her side will give falsely lower readings. Each centimeter above or below heart level translates to an 0.8 mm Hg change. Thus these readings will naturally differ from those taken when the arm is appropriately at heart level (i.e., woman in supine position with the arm at her side or preferably seated with the arm resting on a standard table top).

In listening to blood pressure readings, the first of at least two consecutive beats should be recorded as phase I, the systolic pressure. The AHA recommends that the onset of phase V (disappearance of sound), marked by the last sound heard, be used for defining diastolic pressure in adults. The onset of phase IV or muffling, sometimes used in the past, is harder to recognize and is subject to greater interobserver and intraobserver variability. The vast body of epidemiologic evidence from clinical trials in nonpregnant populations, linking levels of diastolic pressure to increased cardiovascular disease risk, is based on the phase V criterion. In pregnancy phase V seems to correlate best with intraarterial pressure.[33]

However, the AHA recognized certain conditions in which phase V is absent (i.e., the Korotkoff sounds do not disappear but may be heard until the pressure in the cuff falls to near 0 mm Hg). Among these conditions are high cardiac output states including pregnancy. Also, the WHO has noted that in approximately 15% of pregnant women the diastolic pressure falls to zero before the last sound is heard. Under these conditions, phase IV is a better index of diastolic pressure than is phase V. Both the AHA and WHO advise that if the phase IV (muffled) sounds are heard to zero (absent phase V), both phase IV and phase V readings should be recorded (e.g., 148/84/0 mm Hg). It seems prudent, given that the AHA recommends recording the onset of phase V as the diastolic blood pressure in adults, coupled with the probability of an absent phase V in a certain percentage of pregnant women in whom the onset of phase IV should then be recorded, that personnel should record both readings throughout the duration of the woman's pregnancy starting at the woman's initial prenatal visit. This will help ensure that no information will be lost (e.g., baseline phase IV and V readings) and that subtle changes will be detected.

LETTERS TO THE EDITORS

Discrepancies in hemoglobin levels

To the Editors: Danskin and Neilson (Danskin FH, Neilson JP. Twin-to-twin transfusion syndrome: what are appropriate diagnostic criteria? AM J OBSTET GYNECOL 1989;161:365-9) in reporting 178 consecutive twin pregnancies commented on the difficulties in the diagnosis of twin-to-twin transfusion syndrome. In their article they state that venous blood samples were usually taken from the neonates on the first day of life. We would like to point out that this method may misdiagnose the fetal hemoglobin level.

We recently checked and noted discrepancies in hemoglobin concentrations in blood collected from the umbilical vein at delivery and in the first day of neonatal life. In 12 cases of twin pregnancy umbilical venous cord blood samples were collected at delivery and the hemoglobin estimations were compared with results from venous blood samples taken on the first day of life. Discrepancies were present in 21 of the 24 infants delivered. In the eight neonates delivered small-for-gestational-age (SGA) all had a higher value in the neonatal specimen (mean difference, 34.3 gm/L ± SD 33.0; range, 8 to 108 gm/L; $p < 0.05$). In the neonates delivered appropriate-for-gestational-age (AGA) this difference was not so evident (mean difference, 12.5 gm/L ± SD 15.1; range, 0 to 59 gm/L; $p < 0.005$). In these cases there was an increase in eight, there was no change in three, and there was a decrease in hemoglobin level in five. The mean difference for SGA neonates was significantly higher than that seen in the AGA neonates ($p < 0.05$). We suggest that the hemoglobin changes result from the rapid changes in the state of hydration in the first day of life and these changes are greater in SGA infants.

We conclude that studies that rely on differences in hemoglobin and hematocrit levels to confirm the diagnosis of twin-to-twin transfusion in the presence of monochorionic placentation should be performed on cord blood samples collected at the time of delivery. The data of Danskin and Neilson may not be accurate in diagnosis of the true incidence of twin-to-twin transfusion syndrome.

Warwick Giles, PhD, Brian Trudinger, MD, and Gavin Wilcox, BSc

Department of Obstetrics and Gynaecology, University of Sydney at Westmead Hospital, Westmead, New South Wales, 2145, Australia

Response declined

Cytogenetic uncertainties surrounding the fragile X in Martin-Bell syndrome

To the Editors: The fragile site of Xq27 first noted by Lubs[1] and later appreciated by clinical geneticists for its diagnostic significance in Martin-Bell syndrome, remained a simple curiosity during the early 1970s.[2] In recent years, many laboratories have vigorously tried to modify their methods because specific tissue culture conditions are required for the expression of the fragile site at Xq27. Consequently, many more fragile sites in the human genome have been observed. Telomeric structural changes similar in appearance to the fragile X site on the long arm of chromosomes with identical morphology to the X chromosome have created a perplexing dilemma. These sites have been incorrectly misinterpreted as fragile X in a number of patients. One such example is chromosome 6, which also has a fragile site at q26 and is often a source of error.

There are persons who have a mixed population of cells with fragile site X(q27) and fragile site 6(q26) creating difficulties in the determination of an accurate estimate of the population with fragile X cells.[3] There are some persons who have gone undetected because of an extremely low percentage of cells with fragile site on chromosome X. The task remains arduous because various patients exhibit cells with a large range (1% to 50%) of fragile X. Thus it is suggested that if fragile X occurs in >0.7% of cells in males and >1.5% of cells in females from a single culture, then this probably indicates a specific fragile X expression.[4]

Routinely, the fragile X chromosome has been identified with the G-banding method. However, the harsh treatment-effect of trypsin during G-banding often results in poor morphology of the fragile sites. Consequently, the fragile X chromosome can escape detection, especially in cases with low incidence of expression.[5,6] Alternatively, if only conventional staining (solid stain) is used, chromosome 6 can be misinterpreted as a fragile X.

In our experience the conventional staining method remains highly practical in estimating the incidence of cells with fragile X. Conventionally stained metaphases can be rapidly destained and then restained by the Q-banding technique for positive chromosomal identification of those metaphases with fragile sites (Fig. 1). This confirmative approach is highly efficient, especially in subjects with a very low percentage of cells with fragile X or those who have fragile sites that involve other autosomes. With use of this approach six of 100 cases referred to our laboratory for confirmation were determined to have been erroneously identified as fragile X. A wrong diagnosis will have serious consequences, especially in cases that are identified by prenatal diagnosis, although it is understood that the absence of fragile X does not exclude the possibility of X-linked mental retardation in fetuses.

Veena Sindwani, PhD, and Ram S. Verma, PhD

Division of Genetics, Long Island College Hospital, 340 Henry St., Brooklyn, NY 11201

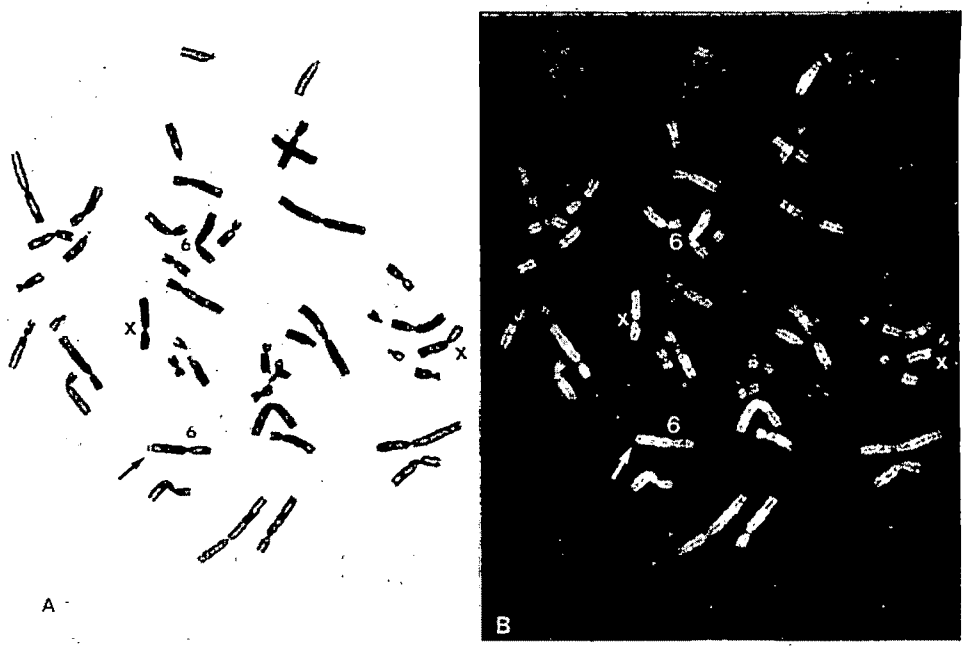


Fig. 1. A, sequentially stained metaphase from female erroneously identified as fragile X by conventional staining. **B**, same chromosome was correctly identified as chromosome 6 by Q-banding technique.

REFERENCES

1. Lubs HA. A marker X chromosome. Am J Hum Genet 1969;1:231-44.
2. Opitz JM, Neri G, Spano LM (eds). X-linked mental retardation. Am J Med Genet 1988;30:1-701.
3. Leversha MA, Webb GC, Pavey SM. Chromosome banding required for studies on X-linked mental retardation. Lancet 1981;1:49.
4. Steinbach P, Barbi G, Boller T. On the frequency of telomeric chromosomal changes induced by culture conditions suitable for fragile X expression. Hum Genet 1982; 61:160-2.
5. Howard-Peebles PN. Chromosome banding in X-linked mental retardation. Lancet 1981;1:494.
6. Soudek D, McGregor T. Source of error in fragile X determination. Lancet 1981;1:556.

Magnesium sulfate is not an ideal anticonvulsant

To the Editors: Dr. Sibai has described magnesium sulfate as an ideal anticonvulsant (Sibai BM. Magnesium sulfate is the ideal anticonvulsant in preeclampsia. AM J OBSTET GYNECOL 1990;162:1141-5). He also implies that magnesium sulfate prevents maternal deaths. The deaths that I have studied from eclampsia have not been related to seizures, but rather to liver rupture or failure, cerebral hemorrhage, and disseminated intravascular coagulation. Some had not received magnesium sulfate, but I doubt that it would have prevented the deaths.

In our hospital, the cesarean section rate was 37.9% in parturients who received magnesium sulfate and 8.3% in those not given the drug. Much of this was because of failed induction of labor in gravida with severe preeclampsia who had an unfavorable cervix. The cases were not comparable, but I am convinced that prophylactic magnesium sulfate leads to a higher cesarean rate than would occur with use of phenytoin.

In one 4-week period, we had a patient convulse who had therapeutic levels of magnesium sulfate and another patient who received phenytoin in appropriate doses. Each responded to the other drug. Whereas magnesium sulfate may be "ideal" as Dr. Sibai has described, it is not always effective.[1]

If there is any pharmacology involved in magnesium sulfate administration, why do we use the same loading dose regardless of maternal size?

I have seen the American College of Obstetricians and Gynecologists reverse themselves on several issues (electronic fetal monitoring, prenatal Rh immune globulin, and fetal scalp sampling) and they may do so again with magnesium sulfate when younger people take the helm. Besides, I doubt whether the American College of Obstetricians and Gynecologists believes that they set the standards of obstetric care.

And finally, I wonder whether Dr. Sibai has been given an intravenous push of 6 gm of magnesium sulfate? At a patient's insistence, I have. After this dreadful experience, even if magnesium sulfate were 100% effective, I would never agree that it is an "ideal" drug, although it may be the best available.

Robert C. Goodlin, MD

Department of Health and Hospitals, City and County of Denver, 777 Bannock St., Denver, CO 80204-4507

REFERENCE

1. Sibai BM, Lipshitz J, Anderson GD, Dilts PV. Reassessment of intravenous MgSO4 therapy in preeclampsia-eclampsia. Obstet Gynecol 1981;57:199-205.

Reply

To the Editors: I thank Dr. Goodlin for his interest in the subject of the use of magnesium sulfate in preeclampsia. It is apparent that he did not get the message conveyed by my manuscript.

The emphasis of the article was on the fact that no other drug has been proved as effective as magnesium sulfate in the prevention of convulsions. I did not imply that the use of magnesium sulfate prevents maternal deaths. Maternal deaths are prevented by the experience of the staff and the availability of the appropriate facilities in a certain institution. The data on mortality were included to show that excellent maternal outcome was achieved in the absence of anticonvulsive agents other than magnesium sulfate. Thus magnesium sulfate is not a bad medicine as shown by this excellent maternal and prenatal outcome.

There are no data to indicate that cesarean section in preeclampsia is usually a result of failed induction. The rate of cesarean section for failed induction in preeclampsia is similar to the respective rate in any pregnancy being induced without the use of magnesium sulfate.

With regard to the efficacy of magnesium sulfate, I did not imply that it is universally effective. In fact, this claim cannot be made for any drug in medicine. However, I noted that the failure rates in the prevention or control of eclamptic convulsions are still superior to any other drug including phenytoin and diazepam. It is important to note that since the publication of my article, three reports appeared in the literature that indicate that magnesium sulfate is the ideal anticonvulsant in eclampsia. One report by Slater et al.[1] found recurrence of convulsions in two of eight women (25%) who received phenytoin.

Dommisse[2] reported on 22 patients with eclampsia who were randomly allocated to receive either intravenous phenytoin ($n = 11$) or magnesium sulfate ($n = 11$). Four of the 11 treated with phenytoin (36%) had further convulsions that subsequently responded to magnesium sulfate, whereas subsequent convulsions did not develop in the magnesium group. Crowther[3] reported on 51 patients with eclampsia who were randomized to receive either magnesium sulfate ($n = 24$) or diazepam ($n = 27$). Recurrent convulsion occurred in five (21%) patients in the magnesium group and seven (26%) patients in the diazepam group. In addition, the author reported that magnesium sulfate has advantages over diazepam for the mother and infant in the treatment of eclampsia.

Dr. Goodlin is worried about the painful side effects of magnesium sulfate as evident from his experience receiving it. No, I have not been given an intravenous push of 6 gm of magnesium sulfate, and will not take it to experience the effects. However, I will receive it if it ever becomes possible for me to become pregnant and develop either preterm labor or preeclampsia.

Baha M. Sibai, MD

Department of Obstetrics and Gynecology, 853 Jefferson Ave., Suite E102, Memphis, TN 38103

REFERENCES

1. Slater RM, Wilcox FL, Smith MD, Maresh MJA. Phenytoin in preeclampsia [Letter]. Lancet 1989;2:1224.
2. Dommisse J. Phenytoin sodium and magnesium sulfate in the management of eclampsia. Br J Obstet Gynaecol 1990;97:104-9.
3. Crowther C. Magnesium sulfate versus diazepam in the management of eclampsia: a randomized clinical trial. Br J Obstet Gynaecol 1990;97:110-7.

Parameters of hypothalamic aging

To the Editors: We read with great interest the study by Alexander et al. (Alexander SE, Aksel S, Hazeton JM, Yeoman RR, Gilmore SM. The effect of aging on hypothalamic function in oophorectomized women. AM J OBSTET GYNECOL 1990;162:446-9) and wish to comment on a critical issue concerning the study design and conclusions. Although the question posed by the authors is important, the study design failed to demonstrate their conclusions because the parameters assessed were not truly representative of hypothalamic aging. It is misleading to conclude from the data presented that basal, unstimulated levels of gonadotropins are representative of parameters of aging of the hypothalamic-pituitary system per se, in either rats or women.

More specifically, in reviewing the literature that examines the issue of hypothalamic aging it is apparent that aging of the reproductive system in female rats is characterized by a general decline in the frequency of regular estrous cycles. Moreover, it has been elegantly shown by Phyllis Wise that middle-aged rats (9 to 12 months old) have comparable basal concentrations of luteinizing hormone and follicle-stimulating hormone with young rats (3 to 4 months old), but show a significant and dramatic difference in the timing and amplitude of preovulatory gonadotropin surges in response to physiologic amounts of estradiol.[1] Thus it is believed that alterations in the positive feedback action of estradiol occur concurrently with the transition of regular to irregular estrous cyclicity and that these alterations represent parameters of aging of the female rat hypothalamic-pituitary axis. Therefore to extrapolate from available data about rats to women one would not expect a demonstrable difference of basal luteinizing hormone and follicle-stimulating hormone pulsatility between oophorectomized women of different ages. Basal levels are not representative of aging hypothalamic function, although estradiol-induced luteinizing hormore surges may be.

David B. Seifer, MD, and Ervin E. Jones, PhD, MD

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., P.O. Box 3333, New Haven, CT 06510

REFERENCE

1. Wise PM. Estradiol-induced daily luteinizing hormone and prolactin surges in young and middle-aged rats: correlations with age-related changes in pituitary responsiveness and catecholamine turnover rates in microdissected brain areas. Endocrinology 1984;115:801-9.

Reply

To the Editors: We thank Drs. Seifer and Jones for their interest in our study and their comments. Our work solely addressed the issue of hypothalamic aging with respect to gonadotropin-releasing hormone/leutinizing hormone (LH) secretory function in young to old women in total absence of ovarian steroids. Because release of gonadotropin-releasing hormone is reflected in the pulsatile secretion of LH, we specifically searched for age-related changes in the hypothalamus with respect to pulse frequency and amplitude in different age groups. Nowhere in the article did we extrapolate from available data about rats to women and it was of interest to us that an article by Karpas et al., who reported the pattern of pulsatile LH secretion in young, middle-aged, and old castrated male rats, followed our study design.[1] This study showed lower mean LH levels and LH pulse amplitude in the middle-aged and old rats when compared with young rats. The old rats also had significantly lower LH pulse frequency than that of the other two groups. The contention of Drs. Seifer and Jones that changes in basal LH and follicle-stimulating hormone pulsatility are not representative of aging hypothalamic function is certainly not supported by this study.

Their objection to our conclusion is apparently made on the basis of the study by Wise, as quoted in their letter, who studied young and middle-aged rats, ovariectomized and estrogen primed, but not old, anestrous rats, nor their basal gonadotropin pulse patterns. Hypothalamic response to steroids may be altered in aging rats and may possibly be altered in aging women, but we did not attempt to address this issue in our study.

Sam E. Alexander, MD, and Sezer Aksel, MD

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of South Alabama, Mobile, AL 36688

REFERENCE

1. Karpas AE, Bremner WJ, Clifton DK, Steiner RA, Dorsa DM. Diminished luteinizing hormone pulse frequency and amplitude with aging in the male rat. Endocrinology 1983;112:788-92.

Triplet pregnancies—Are we really doing better?

To the Editors: In the past 2 years there have been several articles on multifetal pregnancies, such as the one from Toronto by Dr. Gonen et al. (Gonen R, Heyman E, Asztalos EV, et al. The outcome of triplet, quadruplet, and quintuplet pregnancies managed in a perinatal unit: obstetric, neonatal, and follow-up data. AM J OBSTET GYNECOL 1990;162:454-9). The data presented are very interesting. Especially impressive are the data on survival and long-term prognosis of triplets. The conclusions of the authors were that early diagnosis, meticulous antenatal care, early hospitalization, delivery by cesarean section, and availability of expert neonatologic care are the major determinants of improved outcome. However, in a review of their data and ours[1] it is clear that despite various antenatal efforts, such as early diagnosis, hospitalization and use of tocolysis, in the past 4 decades we could not prolong the mean gestational age at delivery or the average infant weight of triplets. As for early hospitalization, it has been shown that equally good results can be achieved by outpatient management of triplet pregnancies.[2]

We believe that the better prognosis for triplets could be attributed primarily to the great advances in neonatology, whereas our role as obstetricians in these cases is somewhat limited. However, it should not be implicated that advanced obstetric care is futile. We would very much like the authors to share with us their opinion on the issue because we are sure that not all hospitals have the excellent neonatologic facilities and abundance of personnel as the one with which they are associated in Toronto.

Amir Weissman, MD and Peter Jakobi, MD

Department of Obstetrics and Gynecology, Rambam Medical Center, Haifa, Israel 31096

REFERENCES

1. Weissman A, Yoffe N, Jakobi P, Brandes JM, Paldi P, Blazer S. Management of triplet pregnancies in the 80's—are we doing better? Am J Perinatol [In press].
2. Newman RB, Hamer C, Miller MC. Outpatient triplet management: a contemporary review. AM J OBSTET GYNECOL 1989;161:547-55.

Reply

To the Editors: Drs. Weisman and Jacobi make some excellent points. There is no doubt that many of the interventions notated have not been proved prospectively by randomized trials in either singleton or triplet pregnancies. Thus they could be concluded to be contentious. Despite the fact that our review was not set up to evaluate the individual components or weight their importance, I agree that there can be no doubt that quality of neonatal care at the time of delivery and a highly functional team effort in the neonatal intensive care unit are absolutely necessary and quite possibly of the most significance.

However, I think if one looked at outcome over time with specific reference to stillbirths one might find, as we have with singleton pregnancies, that over the past 20 years the stillbirth rate has fallen as well as the neonatal death rate; with a significant incidence of intrauterine growth retardation in triplets (17%) one might also find with the passage of time that there also was a decrease in morbidity. Both of these factors might well relate to the importance of quality obstetric care. Certainly the evidence from isolated neonatal intensive care after neonatal transport, as opposed to the improved neonatal outcome from integrated perinatal intensive care after fetal-maternal transport, would also suggest that the obstetric component is of some significance.

The point with respect to outpatient management versus inpatient management is contentious as well, inasmuch as the outpatient management referred to relates predominantly to monitoring of uterine activity and educational efforts by nurses (as well as frequent hospitalization, when indicated, and tocolytic therapy). As the American College of Obstetricians and Gyne-

cologists pointed out in a recent bulletin, the place of all or any of the above has not been proved in the prevention of preterm delivery. Perhaps with the widespread use of this approach at the present time in North America it will never be able to be proved adequately in hard scientific terms. We all know the difficulties we have with respect to the place and value of electronic fetal heart rate monitoring.

I think the bottom line at the present time is that when one puts the total package together one can get good results with quality care. Patients should be aware of this both at the time of in vitro fertilization and in the discussion of selective reduction before any final decision is made.

J. E. Milligan, MD
Regional Perinatal Unit, Women's College Hospital, 76 Grenville St., Toronto, Ontario, Canada M5S 1B2

One case of 45,X abortus with high CA 125 level

To the Editors: In our report (Check JH, Nowroozi K, Winkel CA, Johnson T, Seefried L. Serum CA 125 levels in early pregnancy and subsequent spontaneous abortion. Obstet Gynecol 1990;75:742-4; submitted for publication July 27, 1989) of the relationship of CA 125 to spontaneous abortion, we included one case of a fetal karyotype of 45,X. This case led us to speculate on the possibility that an elevated CA 125 level could suggest a chromosomal abnormality. This was the only abortus that had been karyotyped in the initial series, and we were prompted to try to karyotype subsequent abortions in an effort to support our hypothesis. The question was, Is this associated only with 45,X or with other aneuploidies? After subsequently finding two additional cases, a trisomy 21 and a trisomy 12 and 13, we submitted a case report, which was published subsequently in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (Check JH, Nowroozi K, Vaze M, Wapner R, Seefried L. Very high CA 125 levels during early first trimester in three cases of spontaneous abortion with chromosomal abnormalities. AM J OBSTET GYNECOL 1990;162:674-5; submitted for publication August 10, 1989), including not only the latter two cases but also the 45,X case. This may have inadvertently given the reader the impression that we have seen two cases of 45,X karyotype with an elevated CA 125 level. We write this letter to clarify that we have observed only one case of 45,X abortus with high CA 125 level.

We have subsequently found other cases that have also had chromosomal abnormalities, including another trisomy 21 and a trisomy 10. We are presently engaged in a prospective study aimed at determining whether elevated CA 125 levels in the early first trimester indicate an increased risk of fetal chromosomal abnormalities or whether the association is merely coincidental.

We apologize for any confusion with the reported case and want to reiterate that we reported only one case of elevated CA 125 and subsequent abortion in a fetus with 45,X karyotype.

Jerome H. Check, MD
7447 Old York Road, Melrose Park, PA 19126

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

Common Problems in Obstetric Care, April 3-6, 1991, Westin Maui, Maui, Hawaii. Sponsored by the Departments of Obstetrics and Gynecology, Cornell University Medical College–The New York Hospital and The University of Hawaii Medical Center. For information contact: Colette Carmeris, Course Coordinator, Department of Obstetrics and Gynecology, NYH-CUMC, 525 East 68th St., New York, NY 10021. Tel.: (212) 746-3058.

Fourteenth Annual Mid-Winter Symposium in OB/GYN, Scottsdale Hilton Hotel, Scottsdale, Arizona, February 27–March 1, 1991. Co-Sponsored by the Phoenix OB/GYN Society and the Department of OB/GYN, Maricopa Medical Center. Contact: The Secretary, Phoenix OB/GYN Society, 511 W. Montebello, Phoenix, AZ 85013. Tel.: (602) 266-6321.

14th Annual Gold Coast Ob/Gyn Conference, "Clinical Trends in Ob/Gyn," will be held February 11-13, 1991, in West Palm Beach, Florida. The course is sponsored by Good Samaritan Hospital of West Palm Beach, Florida, and approved for 12.0 credit hours in Category I. For more information contact Laura J. Lehmann, CME Coordinator, P.O. Box 024308, West Palm Beach, FL 33402. Tel.: (407) 650-6236.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

• Gynecologic Oncologist • Reproductive Endocrinologist • Clinical Geneticist

The Baystate Medical Center is the major provider of tertiary care in Western Massachusetts. It has recently expanded its resources through its designation as the Western Campus for Tufts University Medical School. In response, the Department of Obstetrics and Gynecology is expanding its full time faculty group.

The Department has over six thousand deliveries and three thousand operative procedures. Our staff consists of forty-five community based physicians, five certified nurse midwives, and eleven full time faculty in the fields of Maternal-Fetal Medicine, Reproductive Endocrinology, Gynecologic Oncology and Primary Care. The Department has a major commitment to Student Education and has an independent Resident Education Program with twenty residents. Excellent opportunities exist for basic and clinical investigation in many areas.

Baystate Medical Center offers a salary and academic rank commensurate with experience. The Department also has a practice plan. Send Curriculum Vitae or contact:

Laurence E. Lundy, MD, Chairman, Obstetrics and Gynecology
John Powell, MD, Chief, Gynecologic Oncology
Patrick O'Grady, MD, Chief, Maternal Fetal Medicine
Halina Wiczyk, MD, Chief, Reproductive Endocrinology

Department of Obstetrics and Gynecology
Baystate Medical Center
780 Chestnut Street
Springfield, MA 01107
(413) 784-5457

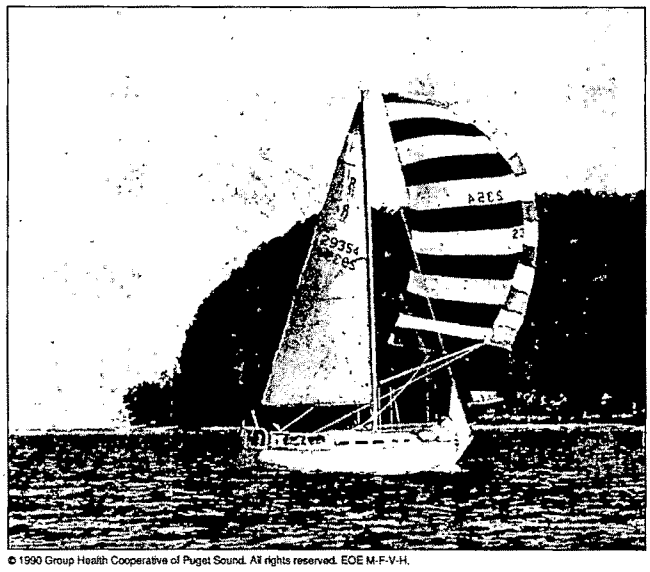
We are an equal opportunity employer.

Baystate Medical Center

THE WESTERN CAMPUS OF TUFTS UNIVERSITY SCHOOL OF MEDICINE

A member of Baystate Health Systems

Look at health care from our point of view.



© 1990 Group Health Cooperative of Puget Sound. All rights reserved. EOE M-F-V-H.

The obstetrician gynecologists who practice at Group Health are here because quality of life is just as important to them as the quality of their work environment.

They enjoy being part of a highly respected health care organization as much as they like living in one of the top areas in the nation.

If you like our point of view, we invite you to call us toll-free at (800) 543-9323 for more information about career opportunities at Group Health.

Group Health Cooperative
of Puget Sound

OBSTETRICAN/GYNECOLOGIST
COLUMBIA, SOUTH CAROLINA

A progressive six man OB/GYN Practice with a Maternal-Fetal Medicine Specialist is seeking an additional partner to practice obstetrics and gynecology.

This practice is based at one hospital, has a new office, two ultrasound machines, and in-office laser surgery. We are very active in high risk pregnancies and operative laparoscopy.

We are looking for a board certified or board eligible, young physician to associate with this practice on a permanent basis. Salary is competitive with comprehensive fringe benefits package.

Association will lead to full and equal partnership. We are seeking a new partner as of July 1, 1991.

For more information, please send a letter and Curriculum Vitae to:

Alexander R. Smythe, II, M.D.
Columbia OB-GYN Associates, P.A.
1333 Taylor St., Suite 5-F
Columbia, South Carolina 29201

OB/Gyn or Perinatologist

Faculty appointment based full-time at affiliated hospital (500 beds, 4000 deliveries, Level III nursery) in moderate-sized city. Primarily obstetrics but little night/weekend call. Excellent opportunity for board certified/eligible generalist or perinatologist to teach & practice in environment conducive to family life. Competitive salary & benefits.

Send CV to:

Charles B. Hammond, MD
Chairman
Department of OB/Gyn
PO Box 3853
Duke University Medical Center
Durham, North Carolina 27710
or FAX 919-684-6161

AA/EOE

MATERNAL-FETAL MEDICINE
CREIGHTON UNIVERSITY

Perinatologist needed to join two man Division in growing, rejuvenated Department at a major Jesuit university. Excellent institutional support, two teaching hospitals with good variety of challenges and 4000 deliveries annually. Affiliation with Boys Town National Research Hospital. Metropolitan center with pleasant weather variety, comfortable lifestyle, and low crime rate. Rank and role in the Division negotiable and dependent on experience, interests, and goals to be mutually established. Highly competitive salary and benefits.

Contact:

Richard P. Perkins, M.D.
Professor of Obstetrics and Gynecology
Director of Obstetric Programs
601 N. 30th Street
Omaha, NE 68131

ELKO REGIONAL MEDICAL CENTER

762 - 14th Street Elko, Nevada 89801 (702) 738-3111

OBSTETRICIAN/GYNECOLOGIST

Nevada's oldest multi-specialty clinic seeks BE/BC OB/GYN to join two other OB-GYN's and Nurse Midwife in very busy practice. Lucrative opportunity for full range of specialty practice. All fee for service with excellent collection ratio. Guaranteed salary for six months leading to full partnership. Comprehensive benefit package including malpractice insurance. Elko is a thriving community surrounded by mountains and wilderness areas. Recreation year round. Please send C.V. to:

Cherie Atwood, Administrator
Elko Regional Medical Center
762-14th Street
Elko, Nevada 89801
(702) 738-3111

FACULTY POSITION
DIVISION OF MATERNAL-FETAL MEDICINE
ALBANY MEDICAL COLLEGE

Rapidly expanding division in our Department of Ob/Gyn needs a third board eligible/certified individual. Candidates should have a strong background in obstetric ultrasound as well as a commitment for medical student and resident education. Protected time for research and other academic pursuits guaranteed. New facility nearing completion. Academic rank in accordance with experience. Competitive salary plus attractive faculty incentive plan provided.

Albany, capital of New York State, offers major cultural and recreational opportunities as well as a variety of life styles in a sane environment. INTERESTED CANDIDATES SHOULD SEND A LETTER OF INTEREST WITH CV TO:

Myron Gordon, M.D.
Chairman, Department of OB/GYN
Albany Medical College A-74
47 New Scotland Avenue
Albany, New York 12208
OR
Paul J. Weinbaum, M.D.
Head, Division of Maternal-Fetal Medicine
Department of OB/GYN A-133

ATLANTA

Multi-specialty group seeks BC/BE Obstetrician/Gynecologist to join sole OB/GYN in 44 physician group. Practice includes primary care OB/GYN and some reproductive endocrinology, laser infertility surgery, hysteroscopy, and oncology. A special interest in high risk OB desirable. Forward inquiries and C.V. to:

Ms. Billie Clary
Administration
100 Tenth Street, N.W.
Atlanta, Georgia 30309

BRIGHAM AND WOMEN'S HOSPITAL

Obstetrician/Gynecologist

The Brigham and Women's Hospital, a major teaching hospital of Harvard Medical School, seeks several obstetrician/gynecologists to join a busy full-time faculty group practice in general obstetrics and gynecology. The practice is located in a private setting near the hospital.

Applicants should be Board-certified/eligible.

Academic appointment at Harvard Medical School will be granted.

Offers competitive economic package and generous fringe benefits.

Commitment to clinical care and teaching are essential.

Send inquiry and curriculum vitae to:
Kenneth J. Ryan, M..D., Chairman
Department of Obstetrics and Gynecology
Brigham and Women's Hospital
75 Francis Street, ASB1-3-073
Boston, Massachusetts 02115

The Brigham and Women's Hospital is an affirmative action, equal opportunity employer.

CHAIR, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

The Allentown Hospital–Lehigh Valley Hospital Center (TAH-LVHC), an 830-bed regional tertiary care teaching hospital is seeking an OB/GYN Chair. The hospital has 33 active OB/GYN staff physicians, including a gynecologic oncologist and reproductive endocrinologist. The current Chair, who will be leaving at the end of this year, is a perinatologist. The hospital is currently recruiting a maternal-fetal subspecialist. TAH-LVHC is a regional Level III Perinatal Center and a clinical campus of Hahnemann University in Philadelphia. Of over 3,300 births in 1989, 42% were in the high-risk category. The department is supported by a 20-bed Level III NICU and has an active OB/GYN clinic which experienced over 8,000 visits in 1989.

NEWLY-CREATED POSITION OF VICE CHAIR/DIRECTOR OF EDUCATION

In response to the continuing growth of the community and the expansion of the OB/GYN Department, TAH-LVHC is seeking its first full-time OB/GYN Vice Chair/Director of Education. The hospital has a fully-approved free-standing Residency Program with eight American-trained residents. A total of 70 residents and 400 medical students are trained at the hospital each year.

The hospital is located in Allentown, a cosmopolitan city one hour north of Philadelphia and one and one-half hours west of New York City. Interested board-certified candidates may send a curriculum vitae, in confidence, to **Headley S. White, Jr., M.D., Sr. V.P., Medical and Academic Affairs, c/o HealthSearch, 50 College Drive, Suite 323, Allentown, PA 18104. (215)778-7993.**

Academic Gynecologic Oncologist

The Dartmouth-Hitchcock Medical Center is searching for a Board Certified Gynecologic Oncologist at the Associate Professor Level. He/She must be an accomplished investigator and be interested in developing a medical center and state-wide academic and clinical program in gynecologic malignancy. Please send CV with names and addresses of three references to:

L. Herbert Maurer, M.D., Chairman
Gynecologic Oncologist Search Committee
Hematology/Oncology
Dartmouth-Hitchcock Medical Center
2 Maynard Street
Hanover, NH 03756

Dartmouth-Hitchcock Medical Center is an Affirmative Action/Equal Opportunity Employer.



Dartmouth-Hitchcock Medical Center

The Hitchcock Clinic
Hanover, New Hampshire

PERINATOLOGIST

Outstanding practice opportunity for a BC/BE perinatologist to join an established practice at Iowa's largest private teaching medical center. The hospital, a state designated Level III Perinatal Center, averages over 2,000 deliveries annually and has an active maternal and neonatal transport system. The medical center's Level III Neonatal Intensive Care Unit is staffed by four board certified neonatologists. Complete laboratory facilities are available including cytogenetic analysis of both amniotic fluid and trophoblastic tissue. The Antepartum Testing Unit is equipped with computer-based fetal monitors and a color flow Doppler ultrasound unit. Excellent financial and lifestyle opportunities are available to the qualified candidate. Please call 515/283-5911 or send CV to Mariann Clark, Medical Search Consultant, 1200 Pleasant Street, Des Moines, IA 50309.

SOUTHEASTERN KANSAS
BUILD A SUCCESSFUL PRACTICE

Progressive, 188-bed medical center, located in a beautiful, Southeastern Kansas university town, has great need for another OB/GYN. Because of over 500 deliveries in the service area, the two OB/GYN's and Family Practice physicians cannot meet the demand for both Obstetrics and Gynecology. The 28-physician medical staff will welcome you and the hospital will do everything possible to help establish your practice. Build this fine practice while enjoying a quality lifestyle in a college town. Outstanding financial package.

Send CV or call Mark Hanna at:

JACKSON AND COKER, INC.
115 Perimeter Center Place
Suite 380 AJOGO
Atlanta, GA 30346
1-800-544-1987

FACULTY POSITION IN OB/GYN
MATERNAL-FETAL MEDICINE

Wright State University School of Medicine, Department of Obstetrics and Gynecology invites applications for a full-time faculty position in maternal-fetal medicine at the Assistant/Associate Professor level. Primary faculty duties will consist of medical student and resident teaching and supervision. The candidate must be licensable to practice medicine in Ohio. Other qualifications include board eligibility or certification in obstetrics and gynecology and completion of a fellwoship in maternal-fetal medicine. Women and minorities are encouraged to apply. Please send curriculum vitae and the names of three references to:

Melvin G. Dodson, M.D., Ph.D.
Nicholas J. Thompson, Professor and Chair
Department of Obstetrics and Gynecology
Wright State University School of Medicine
One Wyoming Street
Dayton, Ohio 45409

All applications received by December 15, 1990, will be considered; if the position is not filled therefrom, applications will be considered as received until the position is filled. Wright State University is an Affirmative Action and Equal Opportunity employer.

Loyola University Chicago Faculty Positions

Maternal Fetal Medicine

Board Certified/Board eligible physician to join 5 person academic group perinatal practice as a full time faculty member in the Department of Obstetrics and Gynecology.

OB/GYN Generalist

Outstanding opportunity for OB/GYN Board eligible/Board Certified physician who is interested in a full time academic position.

Contact:

John H. Isaacs, M.D., Chairman
Obstetrics and Gynecology Recruitment Committee
Loyola University Chicago
2160 South First Avenue
Maywood, Illinois 60153
(708) 216-3383

OBSTETRICIAN/GYNECOLOGIST

Board Certified or Eligible

Lutheran General Hospital, a 713-bed teaching hospital affiliated with the University of Chicago, Pritzker School of Medicine, is recruiting a generalist to join our hospital-based clinical faculty to share in patient care and education. There are more than 5000 deliveries annually, and over 1000 major gyn cases. Our Perinatal Center is the regional referral facility for a service area with more than 12,000 births, and will soon be part of the University of Chicago Perinatal Network. A completely new labor and delivery floor oriented towards the LDR concept opens next fall, and will provide optimal space and services for high risk and normal obstetrical patients. We have an active Ambulatory Surgery Unit, and committed operating rooms for major inpatient surgery. Inpatient and outpatient laser and pelviscopy instrumentation is available, and our new IVF program has recently delivered its first baby. Our Perinatal services are supported by an active Genetics program, and a large NICU and Neonatalogy Faculty. Our hospital-based faculty now includes five Generalists, four Perinatalogists, two Gyn Endocrinologists, and two Gyn Oncologists. All faculty members will have clinical appointments at the Pritzker School of Medicine.

Clinical responsibilities involve the care of ambulatory and inpatients as part of the group, with involvement of residents and students. Our patient mix is largely private or managed care. Additional educational responsibilities include clinical and didactic teaching of Ob/Gyn, Family Practice, and Surgical residents, and medical students at 3rd and 4th year levels. Interest and experience in clinical research is valued, and matches our plans for program development. The position is available now, and we hope to fill it quickly.

Our hospital is in Park Ridge, an attractive suburb northwest of Chicago. We offer a competitive salary and benefits package that is commensurate with experience and qualifications. Please send your C.V. to: **John W. Grover, MD, FACOG, Chairman, Department of Obstetrics and Gynecology, 1775 Dempster St., Park Ridge, IL 60068, or call directly at 708/696-6994.** Equal Opportunity Employer.

Lutheran General Hospital

The Mount Sinai Medical Center of New York

Gynecologic Oncologist

An outstanding career opportunity exists within our Division of Gynecologic Oncology for an additional faculty member at the assistant or associate professor level.

The Mount Sinai Medical Center comprises the Mount Sinai School of Medicine and the Mount Sinai Hospital, a 1200-bed newly refurbished state-of-the-art comprehensive teaching center in New York City. It is affiliated with The City Hospital, Elmhurst Queens and two private hospitals.

The department sponsors a fully accredited residency program with twenty-four residents and fellowship programs for specialty certification in Maternal Fetal Medicine, Reproductive Endocrinology and Gynecologic Oncology.

We seek an individual who is interested in a career in academic medicine, becoming actively involved in research, teaching and patient care. We offer a highly competitive benefit package including professional liability coverage, medical and dental coverage, pension, continuing education, vacation, sick time, as well as housing assistance.

For consideration, please direct your letter, curriculum vitae and the names of three references to: Carmel J. Cohen, MD, Director, Gynecologic Oncology, Dept of Obstetrics, Gynecology, & Reproductive Science, Box 1175, One Gustave L. Levy Place, New York, NY 10029 -6574. An Equal Opportunity Employer.

MOUNT SINAI

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

BC/BE Obstetrician/Gynecologist sought to join small congenial clinic setting within established pre-paid multispecialty group. Practice consists of general obstetrics and gynecology, high risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser are welcomed. Association with resident staff and teaching opportunities available.

Kaiser Permanente offers competitive salaries and excellent benefits including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE

KAISER PERMANENTE
Good People. Good Medicine.

OBSTETRICS & GYNECOLOGY

Obstetrician/Gynecologist needed to join a five physician department at a major regional multispecialty clinic in Billings, Montana. Clinic setting includes all specialties and is adjacent to an outstanding tertiary care referral hospital. Excellent opportunity for a top notch board eligible or board certified Obstetrician/Gynecologist looking for a challenging practice in a geographic area offering unlimited recreational potential. Those interested send CV to:

John W. Heizer, M.D.,
Medical Director
The Billings Clinic
P.O. Box 35100
Billings, Montana 59107-5100



BRIGHAM AND WOMEN'S HOSPITAL



Perinatologist

The Brigham and Women's Hospital, a major teaching hospital of Harvard Medical School, seeks a full-time perinatologist to join the Division of Maternal-Fetal Medicine.

Applicants should be board-certified or active candidates for certification in Obstetrics and Gynecology and Maternal-Fetal Medicine.

Academic rank will be commensurate with experience and achievements.

Commitment to clinical care, research, and teaching are essential.

Send inquiry and curriculum vitae to:

Michael F. Greene, M.D.
Director, Division of Maternal-Fetal Medicine
Department of Obstetrics and Gynecology
Brigham and Women's Hospital
75 Francis Street, ASB1-3-078
Boston, Massachusetts 02115

The Brigham and Women's Hospital is an affirmative action, equal opportunity employer.

Life.

It's our life's work.

If your mission in life is to practice the ideals of medicine without administrative demands, we invite your inquiry.

Group Health Plan in St. Louis is a staff model HMO that promotes the ideals of private practice while freeing doctors from the financial baggage of the private practitioner.

Group Health Plan isn't for every physician. Just for those few who want to practice medicine without compromise.

For an interview please talk with the Medical Recruiter at (314) 993-5955. Or write Group Health Plan, 11475 Olde Cabin Road, St. Louis, Mo. 63141. An equal opportunity employer.

GROUP HEALTH PLAN

Faculty Position
The University of Illinois College of Medicine at Peoria*
GYNECOLOGIC ONCOLOGY

A full-time faculty position is available for a physician with board certification or eligibility by the American Board of Obstetricians and Gynecologists and special competence in gynecologic oncology. Candidates should be interested in clinical care, medical student and resident education, and developing a regionalization program for gynecologic oncology. Competitive compensation and excellent benefit package. Applications will be considered until the position is filled. Send inquiry and curriculum vitae to:

Thomas L. Gross, M.D.
Associate Professor and Chairman
Department of Obstetrics and Gynecology
The University of Illinois
College of Medicine at Peoria
One Illini Drive, Box 1649
Peoria, Illincis 61656

**The University of Illinois is an Equal Opportunity/Affirmative Action Employer*

WORCESTER, MASSACHUSETTS
OB/GYN ASSOCIATE BE/BC

Join Established Busy 2 Doctor Group Affiliated with Major Teaching Hospital/Urban to Rural Lifestyle with Excellent Educational Systems. 3550 square foot medical office connected to the hospital. State of the art facilities including: 12 exam rooms, treatment/procedure room, and sophisticated ultrasound equipment.

EXCELLENT COMPENSATION PACKAGE INCLUDES:

- Salary
- Malpractice
- Paid CME & Vacation
- Relocation Expenses
- And Much More

Send or FAX CV To:

Judy Goodney
Director of Professional Relations
Central Massachusetts Health Care, Inc.
Mechanics Tower
100 Front Street, Suite 300
Worcester, MA 01608
(508) 798-8667 FAX (508) 798-3069

OB-GYN

70-Doctor multispecialty group seeking a board certified/eligible Obstetrician-Gynecologist by June 1991 to associate with 4 MDs in an established practice. Well-equipped offices in a modern expanding facility. Big-10 University community of 100,000, superb cultural advantages, ideal for family. Opportunity for medical school affiliation. Excellent salary and benefits with early associateship. Reply with CV:

Ronald H. Deering
101 West University Avenue
Champaign, IL 61820

CLEVELAND CLINIC FLORIDA
GYNECOLOGIST

Cleveland Clinic Florida, a multispecialty group practice, has openings for two Board Certified or Board Eligible Gynecologists, a generalist and an infertility specialist. Obstetrics is not currently being practiced at CCF. It is desirable that both generalist and subspecialist have an area of subspecialty interest they have developed or are developing. Participation in research and education is strongly encouraged. Candidates must be eligible to be licensed in the state of Florida.

Please send CV's to:

Johna D. Lucas, M.D.
Department of Gynecology
Cleveland Clinic Florida
3000 West Cypress Creek Road
Ft. Lauderdale, FL 33309

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting for positions in our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

REPRODUCTIVE ENDOCRINOLOGIST

• SAN JOSE •

We are currently seeking a Reproductive Endocrinologist to establish and run a reproductive endocrinology subdivision in the department. Duties would include the full spectrum of reproductive endocrinology, outpatient and surgical management of infertility. Fellowship certification would be preferred. The department currently has 17 physicians and is still growing. We offer a generous benefits package including full coverage malpractice insurance. San Jose has beautiful weather and excellent access to Bay Area recreational and cultural activities.

For more information, please contact: Jerome L. Nehemiah, M.D., Chief, Department of OB/GYN, The Permanente Medical Group, Inc., 260 International Circle, Dept. AJOBG, San Jose, CA 95119, or call collect (408) 972-6180. EOE



KAISER PERMANENTE
Good People. Good Medicine.

Finger Lakes Region
Ithaca, New York

Unique opportunity for patient oriented physician in comprehensive OB-GYN service, building on established patient base of 6,000 patients. To include out-patient gynecology, obstetrics, surgery, infertility, and abortions. First call protection from nurse-midwives. Progressive university community (home of Cornell University) offers cultural attractions of a city with the benefits of small town living. Competitive salary, excellent benefits including malpractice. Summer/Fall 1991.

Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.

Planned Parenthood®
of Tompkins County

Board Certified/ Board Eligible

OB/ GYN

Exciting opportunity in fastest growing city in metropolitan New Orleans area. Outstanding opportunity to join prestigious multi-specialty clinic. Exceptional income potential.

Send CV to:
Preston Thompson
180 West Esplanade
Kenner, LA 70065

OB-GYN

BC/BE OB-GYN sought for full service, 260-bed community hospital in San Francisco's dynamic South of Market area.

- Excellent opportunity to join busy, growing practice.
- Competitive salary and benefits package.

ST. LUKE'S HOSPITAL

Send C.V. to:

Walter Kopp
St. Luke's Hospital
3555 Army Street
San Francisco, CA 94110
(415) 641-6543

OBSTETRICIAN GYNECOLOGIST

St. Vincent's Medical Center, affiliated with Yale University School of Medicine and New York Medical College, is currently seeking a full-time Board Certified/Eligible Obstetrician Gynecologist to provide general in-patient and out-patient OB/GYN care for hospital service patients. This responsibility will be shared with another full-time Board Certified physician already on staff. The opportunity for limited private practice is available with appropriate space, equipment and support provided.

Excellent opportunity for individual desiring well compensated position with no starting fees or overhead concerns. Full salary with excellent benefits. Excellent hospital facilities with perinatology and neonatology services on site.

Please send curriculum vitae to:

Paul R. Ramirez, M.D.
Chairman
Department of Obstetrics and Gynecology
St. Vincent's Medical Center
2800 Main Street
Bridgeport, Connecticut 06606
(203) 576-5466

KAISER PERMANENTE

OBSTETRICIAN-GYNECOLOGIST

The Permanente Medical Group, Inc., of San Jose, California is looking for BC/BE physicians for immediate openings in the OB/GYN Department. Positions available either at the main facility in San Jose, or at satellite medical offices in Gilroy. The young, dynamic, department is still growing and currently has seventeen physicians and two nurse practitioners. Our outstanding benefits package includes full coverage malpractice insurance. San Jose and Gilroy share excellent access to Bay Area cultural and recreational activities.

Recent salary advances allow extremely competitive compensation starting with the first year of employment.

For more information, please contact:

Jerome L. Nehemiah, M.D.
Chief, Department of Ob/Gyn
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119
or telephone: 408-972-6180 (collect)

OBSTETRICIAN/ GYNECOLOGIST

Immediate opening for a Board Certified/Eligible physician to work with 5 others at a well-established, growing HMO. Rochester Medical Group is a multispecialty medical group practice serving 45,000 patients at multiple sites. Clinical faculty appointment at the University of Rochester is available with this position. Competitive salary/ benefits with opportunities for research and resident teaching. Send resume to:

Patricia Hosking
Rochester Medical Group, PC
1800 Hudson Avenue
Rochester, NY 14617

LAS VEGAS—Seeking highly qualified board certified/eligible Ob/Gyn physicians to join our 60 physician multi-specialty group. Practice in America's Vacationland—mountain sports, water sports, world class entertainment. Enjoy affordable housing, no state income tax, excellent schools/colleges. High starting salaries, plus bonuses, no investment required. Benefits include malpractice insurance, paid vacation, relocation expenses, health and life insurance, retirement program, continuing medical education, dues, subscriptions, etc. Top tertiary hospitals.

If interested, call me at (702) 877-8638 or send your Curriculum Vitae to: Jerry Reeves, M.D., President, Southwest Medical Associates, P.O. Box 15645, Las Vegas, NV 89114.

OB/GYN BC/BE two positions available in Oklahoma for hospital based practice. New, fully equipped, LDRP facility, with central fetal monitoring, telemetry, dedicated staff and 24 hour obstetrical anesthesia and family prenatal classes. Level II nursery, strong Pediatrician support. Progressive community with colleges, symphony and cultural opportunities. Malpractice rates reasonable. Salary and benefits guaranteed. Reply with CV to Medical Services, Room 3129, P.O. Box 205, Oklahoma City, OK 73101.

OBSTETRICIAN GYNECOLOGIST

Western Massachusetts staff model Health Maintenance Organization is seeking two full-time, Board Certified/Board Eligible Obstetrician/Gynecologists to join our expert staff of five physicians and five midwives.

Our multi-specialty group practice employs 70 full-time physicians and provides care for a membership of over 65,000 patients in our modern suburban facilities. Inpatient care is provided at a 900-bed Tufts affiliated teaching hospital.

Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118 or call (413) 594-3111.

MedicalWest
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

The **Michael Reese Health Plan,** a large well-established HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching. All specialty facilities available.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants shou'd address inquiries to:

Alfred Kendrick, M.D.
Medical Affairs
MICHAEL REESE HEALTH PLAN
2545 King Drive, Chicago 60616
or call (312) 808-4551

Michael Reese Health Plan
an equal opportunity employer m/f/h

OBSTETRICIANS & GYNECOLOGISTS

MOUNT SINAI SCHOOL OF MEDICINE

Affiliation has excellent opportunities at Elmhurst Hospital Center serving western Queens community. Board certified/Board eligible candidates will join team-oriented approach providing healthcare to challenging patient mix in culturally diverse environment, located minutes from midtown Manhattan. Responsibilities include primary clinical care; routine/high-risk obstetrics; resident, fellow, and student teaching.

Academic rank granted by School of Medicine based on individual credentials. Excellent salary and benefits package. Participation in successful physician private practice plan. Send c.v. or call: **Jasmin Moshirpur, MD, Director of Obstetrics & Gynecology/Associate Dean-Elmhurst Affairs, Room #C8-13, (718) 830-1340/1348.**

MOUNT SINAI SERVICES

MSM
MOUNT SINAI SCHOOL OF MEDICINE OF THE CITY UNIVERSITY OF NEW YORK

Elmhurst Hospital Center
79-01 Broadway
Elmhurst, NY 11373
An equal opportunity employer

PERINATAL ASSOCIATES OF NORTHERN CALIFORNIA

Are you a clinical perinatologist? We are three perinatologists and one geneticist-OB/GYN looking for one more perinatologist who: desires superior environment for patient care, in a private, modern, level 3 facility doing 6,000 deliveries a year with superior perinatal outcome; likes personal, direct hands-on patient care; needs PUBS, IUTxF, CVS and complicated pregnancies, level 2 ultrasound; demands partners, not superiors; wants to live in clean, warm, not humid, snow-free (but 60 minutes from skiing) Sacramento, California; feels one's family has priorities, and is committed to the private practice of medicine. If really interested call (916) 733-1755, extension 2773.

Hanns C. Haesslein, M.D.
Perinatal Associates
of Northern California
5301 F. Street
Sacramento, California 95819

Ambulatory Gynecologist

Marshfield Clinic's Department of OB/GYN is expanding and is seeking a board-certified obstetrician/gynecologist for a new Ambulatory Gynecology position. Primary responsibilities include walk-in ambulatory patients, performance of diagnostic office procedures, directing the departments' physician extender clinic, and initial infertility evaluations. No OB or GYN call; no obstetrical care. Marshfield Clinic is a 350 physician multi-specialty private group located in a thriving family-oriented community in central Wisconsin. Please send curriculum vitae to:

George L. Hill, M.D.
Chairman OB/GYN Department
1000 North Oak Avenue
Marshfield, WI 54449
or call collect
(715) 387-5161

Marshfield Clinic

OBSTETRICIAN/ GYNECOLOGIST

Opportunity for a qualified OB/GYN physician to join the staff of the Medical Center of Delaware beginning July 1, 1991.

Responsibilities include treating patients in four area clinics and supervision of residents.

Generous salary and benefits package includes Professional Liability insurance, vacation, health insurance coverage, dental and prescription plans. Newark, Delaware is in close proximity to Philadelphia and Baltimore, sports, cultural events, theatre, and shopping.

Please direct inquiries and C.V. to:

Medical Center of Delaware
c/o Garrett H.C. Colmorgen, M.D.
Obstetrics and Gynecology
Maternal/Fetal Medicine
4755 Ogletown-Stanton Road
P.O. Box 6001
Newark, DE 19718

Northwestern University Medical School Department of Obstetrics and Gynecology Chicago, Illinois

Academic Gynecologist

Responsibilities include development of clinical or basic science research in the field of benign gynecology (interest in the field of urogynecology or infectious diseases is desirable), graduate and undergraduate education, and participation in clinical gynecology service.

Academic rank will be commensurate with qualifications and experience. We encourage individuals interested in career development to apply.

Applications should be sent to:

Lane J. Mercer, M.D.
Chief, Section of Gynecology
Department of Obstetrics and Gynecology
333 East Superior Street, Suite 420
Chicago, Illinois 60611

In order to receive full consideration, application must be received by February 15, 1991.

Northwestern University is an affirmative action/equal opportunity employer. Hiring is contingent upon eligibility to work in the United States.

Ob/Gyn Residency Program Director, Central Texas Medical Foundation at Brackenridge Hospital, Austin, TX. Integrated with St. Joseph Hospital at Houston. Other CTMF residencies in FP, Internal Medicine, Pediatrics and Surgery. Contact or send CV to Earl B. Matthew, M.D., CTMF, 601 East 15th Street, Austin, TX 78701 (512) 480-1872, or Joseph A. Lucci, M.D., St. Joseph, 1819 Crawford, Houston, TX 77002 (713) 757-7478.

OBSTETRICIAN/ GYNECOLOGISTS

Group Health Association, one of Washington, D.C.'s most respected names in the health maintenance field, has immediate and future openings for board certified or eligible Obstetrician/ Gynecologists.

Consisting of nine multi-specialty medical centers and 200 physicians, Group Health's highly skilled support staff serves over 150,000 members. This staff attends to the details of clinical administration, allowing you the freedom to devote your energy to practicing medicine. Further, GHA's challenging work environment offers a diverse patient population and affiliation with the area's finest hospitals.

A generous benefits package including vacation, sick leave and insurance coverage (malpractice, life, retirement, disability and dental) awaits you in return for your expertise. In addition, you can look forward to company-paid hospital and licensing fees, professional society dues, continuing education as well as predictable office hours.

If you would like more information, please send your C.V. to the address below or call Mary Hines, Physician Recruiter, on 1-800-336-1442 or in DC Metro area, (202) 364-7442.

Turner Bledsoe, M.D., Medical Director

Group Health Association

4301 Connecticut Avenue, N.W.
Washington, D.C. 20008

An Equal Opportunity Employer

Two person OB-GYN group, well established, in prestigious Long Island community. Looking for BC/BE Associate leading toward partnership. University and Community hospital affiliations, in-office sonography, cryo surgery, and colposcopy. Excellent salary, benefits, and future.

Send C.V. to:
Marc Harvey, M.D., F.A.C.O.G.
100 Manetto Hill Road
Suite 306
Plainview, NY 11803

GYNECOLOGICAL ONCOLOGIST

Indianapolis, Indiana

Candidate will assume position as Medical Director of Gynecological Service. Must be fellowship-trained in Gynecological Oncology.

Practice at Methodist Hospital of Indiana with 1,120 beds and a tertiary Oncology center. Excellent research opportunities with industrial and cooperative groups includes Molecular Oncology.

Beautiful, environmentally clean area. Easy access to lakeside housing. Low crime rate and low taxes.

Private practice income plus administrative salary commensurate with experience. Send CV or call:

Greg Flowers
JACKSON AND COKER, INC.
115 Perimeter Center Place
Suite 380 AJOGO
Atlanta, GA 30346
1-800-544-1987

OB/GYN

Free yourself from the business aspects of private practice and enjoy the visibility and advantages of a multi-specialty prepaid group practice in SUBURBAN BOSTON. Experience reasonable work and coverage schedules. As a full-time member of our respected staff, you will work in one of our modern, well-equipped facilities in north suburban or south suburban Boston. Ancillary services are on-site. Locations are within reasonable distance of the ocean, ski resorts, cultural and academic centers. Excellent compensation and fringe benefit package including full malpractice coverage. **Send C.V. to Director of Physician Recruitment, Medical East Community Health Plan, One Worcester Road, Framingham, MA 01701; or call collect (508) 879-0077.**

Medical East™
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

OBSTETRICIAN/ GYNECOLOGIST

Large, independent multi-specialty group in Minneapolis-St. Paul metropolitan area seeks additional BC/BE obstetrician/gynecologists to join challenging, established practice spanning the full range of obstetrics and gynecology. Competitive salary and benefit package. Send inquiries and curriculum vitaes to:

Nancy Borgstrom
Administration
Aspen Medical Group
1020 Bandana Boulevard West
St. Paul, MN 55108
(612) 641-7185

Equal Opportunity Employer

Searching for an academic position? Here's an easy way.

Academic Physician Services has established a national clearinghouse for academic physicians. In addition to our bimonthly publication, we offer continuous updates of academic positions in your specialty.

A comprehensive resource.

Medical schools and teaching hospitals throughout the U.S. list their positions in *Academic Physician.* This bimonthly directory is sent free of charge to all academic physicians, post-doctorate fellows, and senior residents, and is filled ONLY with academic positions listed by subspecialty and rank.

Equal Opportunity.

We encourage qualified women and minority physicians to use our service. All medical schools and hospitals listing with *Academic Physician* are equal opportunity employers.

Continuous updates.

New positions arrive every day. To receive the most up-to-date listings in your specialty and to assure your position on our subscription list, call today: (916) 939-4242. It's the easy way.

907 Embarcadero, Suite 1
El Dorado Hills, CA 95630
FAX (916) 939-4249
PHONE (916) 939-4242

ACADEMIC **PHYSICIAN** SERVICES

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

Seeking a BC/BE Obstetrician/Gynecologist to provide outpatient care in our Pleasanton medical office building and inpatient care at the Kaiser Hospital/Walnut Creek. Position involves supervision of NPs. Academic affiliation available. Enjoy easy access to the recreational and cultural activities of the San Francisco Bay area. We offer competitive salary and an excellent benefits package including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Patrick Golden, M.D., The Permanente Medical Group, Inc., 5600 Stoneridge Mall Road, Pleasanton, CA 94588. EOE

KAISER PERMANENTE
Good People. Good Medicine.

OB/GYN—BC/BE for large established northeastern fee-for-service multispecialty group to join active 3 member Department of OB-GYN. Beautiful suburban NYC setting and academic affiliation with one of NY's finest medical schools. Excellent salary leading to partnership. Position for July 1991 or sooner. Send CV to P.O. Box 731 Summit, NJ 07902-0731.

Full Time Faculty Positions

The Department of OB/GYN at Temple University Health Science Center is seeking Board Certified or Board Eligible Obstetrician/Gynecologists in the following areas:

MATERNAL-FETAL MEDICINE
GYNECOLOGIC ONCOLOGY
REPRODUCTIVE ENDOCRINOLOGY
GENERAL OBSTETRICS & GYNECOLOGY

Clinical and academic activities are integrated in a department providing tertiary services. Newly opened Temple University Hospital houses a modern Labor and Delivery Suite and large Neonatal Intensive Care Unit. Candidates should have an active interest in Research, Clinical Practice and Resident and Medical Student Education. Salary and academic rank commensurate with experience. Submit C.V. to: **E. Albert Reece, M.D., Chairman, Dept. of OB/GYN, TEMPLE UNIVERSITY HEALTH SCIENCE CENTER, Broad & Ontario Sts., Philadelphia, PA 19140.** Equal Opportunity Employer.

UNIVERSITY

Choose
TEMPLE

OB/GYN CHIEF

The Department of Gynecology and Obstetrics at The Union Memorial Hospital is currently accepting applications for the position of Chief of Gynecology and Obstetrics and director of a fully accredited residency training program. Union Memorial is a 350 bed teaching hospital in Baltimore, Maryland. Applicants must be board certified in OB/GYN with subspecialty boards preferred, have administrative, clinical, teaching, and research experience. For confidential consideration, please send CV to:

Marcella Roenneburg, M.D.
The Union Memorial Hospital
201 E. University Parkway
Baltimore, Maryland 21218

UNIVERSITY OF DUNDEE
Department of Obstetrics and Gynaecology

PART-TIME CLINICAL SENIOR LECTURER

The holder of this post will undertake some of the clinical responsibilities of Professor P W Howie while he is Dean of the Faculty of Medicine and Dentistry. Applicants should be experienced in Obstetrics and Gynaecology and must hold the MRCOG or equivalent qualification. The successful applicant will be awarded 50% of a clinical lecturer's salary (£31,780 per annum) and will be free to undertake research in the remainder of his/her time. The post would be ideal for someone wishing to undertake sabbatical leave and able to provide the balance of his/her salary from alternative sources; the appointment would be ideally for a two-year period although applications for a one-year appointment would also be considered. Applicants must be eligible for full registration with the GMC in order to qualify for an associated Honorary Consultant Contract.

The Department of Obstetrics and Gynaecology is part of the Centre for Human Development Research at Ninewells Hospital and Medical School, Dundee, and has an active research programme in perinatal medicine running a successful in-vitro fertilisation research unit.

Further particulars from, and applications in writing with CV and the names and addresses of three referees as soon as possible to:

The Personnel Office,
The University,
Dundee,
DDI 4HN
Scotland.

Please quote reference EST/47/90/AOG

Faculty Positions
The University of Illinois College of Medicine at Peoria*

MATERNAL-FETAL MEDICINE

Four full-time faculty positions are available for physicians with board certification or eligibility by the American Board of Obstetricians and Gynecologists and special competence in maternal-fetal medicine. Candidates should be interested in clinical care, medical student and resident education, and participation in a regionalization program for maternal-fetal medicine. Competitive compensation and excellent benefit package. Applications will be considered until the positions are filled. Send inquiry and curriculum vitae to:

Thomas L. Gross, M.D.
Associate Professor and Chairman
Department of Obstetrics and Gynecology
The University of Illinois
College of Medicine at Peoria
One Illini Drive, Box 1649
Peoria, Illinois 61656

**The University of Illinois is an Equal Opportunity/ Affirmative Action Employer*

CHAIRPERSON OF GYNECOLOGY AND OBSTETRICS
UNIVERSITY OF KANSAS SCHOOL OF MEDICINE-KANSAS CITY

Applications and nominations are being sought for the position of chairperson for a dynamic University Department of 12 full-time clinical faculty, 4 full-time research faculty and 12 residents. The department is located at the medical center and fully integrated into the 474 bed University Hospital function. The candidate must have demonstrated leadership and organizational skills, be an effective clinician and teacher and demonstrate expertise in scholarly activity. M.D. with Board certification in Obstetrics and Gynecology is required. Applications and nominations, including a curriculum vitae should be sent to:

Allen Erenberg, M.D.
Chairman, Obstetrics and Gynecology
Search Committee
Professor and Chairman, Department of Pediatrics
University of Kansas Medical Center
39th and Rainbow Blvd.
Kansas City, Kansas 66103

Candidates will be considered until the position is filled. The University of Kansas is an equal opportunity/affirmative action employer.

THE DEPARTMENT OF OBSTETRICS & GYNECOLOGY at the University of Missouri-Columbia School of Medicine is recruiting for two full-time, tenure-track specialist in Maternal-Fetal Medicine. Candidates should be Board Certified, or active candidates in Obstetrics and Gynecology with Board Certification, or active candidates for Board Certification in Maternal-Fetal Medicine. Rank and salary commensurate with qualifications and experience. Deadline for applications is June 30, 1991. Applicants should contact Dr. James W. Daly, M.D., Chairman, Department of Obstetrics and Gynecology, University of Missouri-Columbia, Columbia, Missouri 65212 or phone (314) 882-6361. The University of Missouri is an Equal Opportunity/Affirmative Action Employer.

FACULTY POSITIONS IMMEDIATELY AVAILABLE
Maternal Fetal Medicine - Reproductive Endocrinology - Obstetrics/Gynecology - Urogynecology

The Department of Obstetrics and Gynecology at the Unversity of South Dakota School of Medicine in Sioux Falls, South Dakota, a delightful city located directly on the Sioux River and serving a tri-state area and one of the best kept secrets in the Midwest, is building a full-time academic department. Qualified individuals to contribute, develop and provide leadership in all aspects of the specialty are actively being sought.

Candidates should have interests in clinical practice activities and medical education. Clinical and/or basic research opportunities are available and encouraged. An opportunity for a creative, motivated individual with a pioneer spirit.

Financial remuneration and incentitves are competitive and commensurate with experience, credentials and rank. BC or BE. Position open until filled.

Please send Curriculum Vitae to: R. H. Wiebe, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, University of South Dakota School of Medicine, 1100 South Euclid Avenue, Sioux Falls, SD 57117-5039, (605) 333-7327.

An Equal Opportunity/Affirmative Action Employer

UTMB University of Texas Medical Branch Galveston, Texas

The Department of Obstetrics and Gynecology is currently recruiting for:

Chief of Gynecology

- Board eligibility or certification in gynecology required.
- Proven record of research and commitment to an academic career.
- Academic rank and salary commensurate with experience and qualifications.

Curriculum Vitae should be sent to:

Garland D. Anderson, M.D.
Jennie Sealy Smith Professor & Chairman
Department of Obstetrics & Gynecology
The University of Texas Medical Branch
Galveston, Texas 77550-2776

UTMB is an equal opportunity M/F/V/H affirmative action employer. UTMB hires only individuals authorized to work in the United States.

UNIVERSITY OF UTAH SCHOOL OF MEDICINE MATERNAL-FETAL MEDICINE

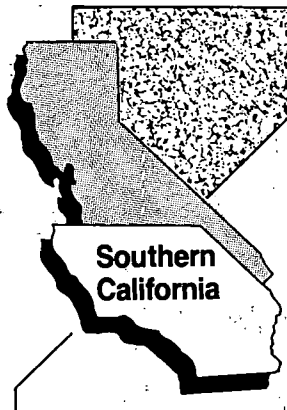
The Department of Obstetrics and Gynecology is seeking two additional faculty members its Division of Maternal-Fetal Medicine. Rank and tenure or clinical track is dependent upon qualifications. The Division (as is also true of the residency program) is an integrated one between the University of Utah Medical Center and the LDS Hospital, a large private hospital three miles from the University. Currently the Division consists of seven faculty members and a fellow. The Division offers a three-year fellowship, and has active programs in genetics high risk obstetrics, (5,600 births per year), prenatal diagnosis, immunology, and ultrasound. Applicants must have a sincere interest in clinical or laboratory research, as well as in teaching and patient care, and should be board certified or active candidates for certification in Maternal-Fetal Medicine. Applications must be postmarked by November 30, 1990. Applications received after that deadline may be considered if no qualified applications have been received by that time. The University of Utah is an Equal Opportunity Employer.

California Opportunity

- Thriving Orange County OB/Gyn Practice seeks first-class BC/BE, OB/Gyn specialist for partnership opportunity.
- Private pay and PPO patient base.
- **Financial assistance available.**

Contact:

M. Christian
Director, Professional Relations
Western Medical Center/Santa Ana
1001 N. Tustin Avenue
Santa Ana, CA 92705
(714) 953-3580



Join The Partnership of Choice

Southern California Permanente Medical Group (SCPMG) is recognized as the nation's largest and most respected physician-managed, multi-specialty medical group.

Our continued growth has created new opportunities for:

OBSTETRICIANS & GYNECOLOGISTS

You'll have a choice of professional environments ranging from a 250-bed community hospital to a major teaching facility in a number of prime Southern California locations. Our active practices require proficiency in modern ultrasound and surgical techniques.

CHIEF OF SERVICE

An excellent opportunity for an experienced OB/GYN practitioner to lead an established and growing practice at our Panorama City Medical Center. You'll be responsible for supervising 20 OB/GYN Physicians, a Perinatologist, 8 Certified Nurse Midwives and 4 Nurse Practitioners. This team of professionals will handle approximately 3000 deliveries per year.

SCPMG provides the freedom, technology and resources to focus on quality patient care and the collaborative support of knowledgeable colleagues. **We also provide a highly competitive salary, paid malpractice and predictable hours.**

KAISER PERMANENTE
Southern California Permanente Medical Group

Partners Practicing Good Medicine

Call or send your CV to:
Irwin P. Goldstein, M.D., Associate Medical Director
SCPMG, Dept. 006
Walnut Center, Pasadena, CA 91188-8013
Or Call: 1-800-541-7946

Mosby Year Book

MEDICAL JOURNALS

Dedicated to Publishing Excellence

SAMPLE COPIES OF JOURNALS ARE AVAILABLE

* AMERICAN HEART JOURNAL
Monthly. Institutions: $125.00; Individuals: $65.00.

* AMERICAN JOURNAL of INFECTION CONTROL
Bimonthly. Institutions: $82.00; Individuals: $23.75.

* AMERICAN JOURNAL of OBSTETRICS and GYNECOLOGY
Monthly. Institutions: $130.00; Individuals: $71.50.

* CLINICAL PHARMACOLOGY & THERAPEUTICS
Monthly. Institutions: $141.00; Individuals: $77.00.

* HEART & LUNG: THE JOURNAL of CRITICAL CARE
Bimonthly. Institutions: $85.00; Individuals: $24.50.

* HEPATOLOGY
Monthly. Institutions: $205.00; Individuals: $150.00.

* THE JOURNAL of ALLERGY and CLINICAL IMMUNOLOGY
Monthly. Institutions: $116.50; Individuals: $53.50.

* JOURNAL of the AMERICAN ACADEMY of DERMATOLOGY
Monthly. Institutions: $130.00; Individuals: $69.00.

JOURNAL of the AMERICAN ACADEMY of PHYSICIAN ASSISTANTS
Eight times a year. Institutions: $50.00; Individuals: $32.00.

* JOURNAL of the AMERICAN SOCIETY of ECHOCARDIOGRAPHY
Bimonthly. Institutions: $56.00; Individuals: $40.00.

* JOURNAL of BURN CARE & REHABILITATION
Bimonthly. Institutions: $46.25; Individuals: $30.00.

* THE JOURNAL of HAND SURGERY (American Volume)
Bimonthly. Institutions: $108.00; Individuals: $46.50.

* THE JOURNAL of HEART TRANSPLANTATION
Bimonthly. Institutions: $72.50; Individuals: $46.00.

* THE JOURNAL of LABORATORY and CLINICAL MEDICINE
Monthly. Institutions: $138.00; Individuals: $73.50.

* THE JOURNAL of PEDIATRICS
Monthly. Institutions: $120.50; Individuals: $59.00.

* THE JOURNAL of THORACIC and CARDIOVASCULAR SURGERY
Monthly. Institutions: $141.00; Individuals: $81.50.

* JOURNAL of VASCULAR SURGERY
Monthly. Institutions: $124.00; Individuals: $67.00.

* OTOLARYNGOLOGY—HEAD and NECK SURGERY
Monthly. Institutions: $143.00; Individuals: $77.00.

* SURGERY
Monthly. Institutions: $123.00; Individuals: $60.00.

* Included in *Index Medicus* J9850FZC

Subscription rates quoted are for USA only. International rates (surface and airmail delivery) available upon request. All subscriptions are for one-year terms. Subscriptions may begin at any time. To order your subscription and for more information, contact:

MOSBY-YEAR BOOK, INC.
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, Missouri 63146 U.S.A.

Call:
(314) 872-8370, ext. 7351
In the U.S., call toll-free:
1-800-325-4177, ext. 7351

OBSTETRICIAN/ GYNECOLOGIST

Board certified or board eligible obstetrician/gynecologist to join two member department in multispecialty clinic. Office ultrasound, colposcopy, and cryosurgery available. Adjacent to 94 bed community hospital with special care nursery.

Worthington, MN is a community of 11,000 located in the highly productive corn belt. Recreational activities include good hunting, fishing, and two 18 hole golf courses. Excellent schools, pollution free environment, with low crime rate make this an ideal community for family living.

Excellent compensation package available. Respond to Mr. John Sieve, Administrator, Worthington Medical Center, 508 10th Street, Worthington, MN 56187 or call 507-372-2921.

PERSONALIZED LIBRARY CASES

Protect your personal copies of AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY in custom made, titled library cases.

Constructed of durable, reinforced binder's board covered in a rich simulated leather, each is designed to hold an entire year's issues. The Journal name is stamped in gold leaf, making the cases attractive additions to any decor, whether on your bookshelf, desk or any location in your home or office.

Only $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.). U.S. funds only.

To charge your order using American Express, Visa, MasterCard or Diners Club, call toll-free: 1-800-972-5858.

Please send me _____ library cases for AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY at $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.).

Name _____
(please print legibly or type)

Address _____
(no P.O. boxes, please)

City/State/Zip _____

PA residents add 6% sales tax

Jesse Jones Industries
499 East Erie Avenue
Philadelphia, PA 19134
Department Code: Mosby

Mosby Year Book

MEDICAL JOURNALS

Dedicated to Publishing Excellence

* AMERICAN HEART JOURNAL
Monthly. Institutions: $125.00; Individuals: $65.00.

* AMERICAN JOURNAL of INFECTION CONTROL
Bimonthly. Institutions: $82.00; Individuals: $23.75.

* AMERICAN JOURNAL of OBSTETRICS and GYNECOLOGY
Monthly. Institutions: $130.00; Individuals: $71.50.

* CLINICAL PHARMACOLOGY & THERAPEUTICS
Monthly. Institutions: $141.00; Individuals: $77.00.

* HEART & LUNG: THE JOURNAL of CRITICAL CARE
Bimonthly. Institutions: $85.00; Individuals: $24.50.

* HEPATOLOGY
Monthly. Institutions: $205.00; Individuals: $150.00.

* THE JOURNAL of ALLERGY and CLINICAL IMMUNOLOGY
Monthly. Institutions: $116.50; Individuals: $53.50.

* JOURNAL of the AMERICAN ACADEMY of DERMATOLOGY
Monthly. Institutions: $130.00; Individuals: $69.00.

JOURNAL of the AMERICAN ACADEMY of PHYSICIAN ASSISTANTS
Eight times a year. Institutions: $50.00; Individuals: $32.00.

* JOURNAL of the AMERICAN SOCIETY of ECHOCARDIOGRAPHY
Bimonthly. Institutions: $56.00; Individuals: $40.00.

* JOURNAL of BURN CARE & REHABILITATION
Bimonthly. Institutions: $46.25; Individuals: $30.00.

* THE JOURNAL of HAND SURGERY (American Volume)
Bimonthly. Institutions: $108.00; Individuals: $46.50.

* THE JOURNAL of HEART TRANSPLANTATION
Bimonthly. Institutions: $72.50; Individuals: $46.00.

* THE JOURNAL of LABORATORY and CLINICAL MEDICINE
Monthly. Institutions: $138.00; Individuals: $73.50.

* THE JOURNAL of PEDIATRICS
Monthly. Institutions: $120.50; Individuals: $59.00.

* THE JOURNAL of THORACIC and CARDIOVASCULAR SURGERY
Monthly. Institutions: $141.00; Individuals: $81.50.

* JOURNAL of VASCULAR SURGERY
Monthly. Institutions: $124.00; Individuals: $67.00.

* OTOLARYNGOLOGY—HEAD and NECK SURGERY
Monthly. Institutions: $143.00; Individuals: $77.00.

* SURGERY
Monthly. Institutions: $123.00; Individuals: $60.00.

* Included in *Index Medicus* J9850FZB

Subscription rates quoted are for USA only. International rates (surface and airmail delivery) available upon request. All subscriptions are for one-year terms. Subscriptions may begin at any time. To order your subscription and for more information, contact:

MOSBY-YEAR BOOK, INC.
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, Missouri 63146 U.S.A.

Call:
(314) 872-8370, ext. 7351
In the U.S., call toll-free:
1-800-325-4177, ext. 7351

SAMPLE COPIES OF JOURNALS ARE AVAILABLE

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION (Required by 39 U.S.C. 3685). 1. Title of Publication: AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY. 1A. Publication number: 019920. 2. Date of filing: September 28, 1990. 3. Frequency of issue: Monthly. 3A. No. of issues published annually: 12. 3B. Annual subscription price: $86.00. 4. Location of known office of publication: 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. 5. Location of the headquarters or general business offices of the publishers: Same as 4. 6. Names and complete addresses of publisher, editor, and managing editor: Publisher—Mosby-Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318; Editor—John I. Brewer, M.D., 710 N. Fairbanks Ct., Suite 8300, Chicago, Illinois 60611; Managing editor—None. 7. Owner: Mosby-Year Book, Inc., a corporation, owner of publication, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. All of the stock of Mosby-Year Book, Inc. is owned by Times Mirror Company, a corporation, Times Mirror Square, Los Angeles, California 90053. 8. Known bondholders, mortgagees, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages or other securities: None. 9. Not applicable to Mosby-Year Book, Inc.

10. Extent and nature of circulation	*Average no. copies each issue during preceding 12 months*	*Actual no. of copies of single issue published nearest to filing date*
A. Total no. copies printed *(net press run)*	22,012	21,500
B. Paid circulation		
1. Sales through dealers and carriers, street vendors and counter sales	None	None
2. Mail subscriptions	20,455	19,868
C. Total paid circulation	20,455	19,868
D. Free distribution by mail, carrier or other means, samples, complimentary, and other free copies	785	580
E. Total distribution *(Sum of C and D)*	21,240	20,448
F. Copies not distributed		
1. Office use, left over, unaccounted, spoiled after printing	772	1,052
2. Returns from news agents	None	None
G. Total *(Sum of E, F1 and 2—should equal net press run shown in A)*	22,012	21,500

11. I certify that the statements made by me above are correct and complete.

Carol Trumbold, *Journal Publisher*

12. In accordance with the provisions of this statute, I hereby request permission to mail the publication named in Item 1 at the phased postage rates presently authorized by 39 U.S.C. 3626.

Carol Trumbold, *Journal Publisher*

INDEX TO ADVERTISERS

Academic Physician Services
Opportunity Available 59A

Albany Medical College
Opportunity Available 49A

Aspen Medical Group
Opportunity Available 59A

Baystate Medical Center
Opportunity Available 47A

Billings Clinic, The
Opportunity Available 52A

Berlex Laboratories, Inc.
Tri-Levlen 40A,41A,42A

Brigham and Women's Hospital
Opportunity Available 49A,52A

Burroughs Wellcome Company
Zovirax 37A,38A

Central Massachusetts Health Care, Inc.
Opportunity Available 53A

Cleveland Clinic Florida
Opportunity Available 53A

Columbia OB-GYN Associates, P.A.
Opportunity Available 48A

Creighton University
Opportunity Available 48A

Dartmouth-Hitchcock Medical Center
Opportunity Available 50A

Duke University Medical Center
Opportunity Available 48A

Elko Regional Medical Center
Opportunity Available 48A

Group Health Association
Opportunity Available 58A

Group Health Cooperative of Puget Sound
Opportunity Available 47A

Group Health Plan
Opportunity Available 52A

Harvey, Marc
Opportunity Available 58A

HealthSearch
Opportunity Available 49A

Jackson and Coker, Inc.
Opportunity Available 50A,58A

Kaiser Permanente
Opportunity Available 62A

Loyola University Chicago
Opportunity Available 51A

Lutheran General Hospital
Opportunity Available 51A

Marshfield Clinic
Opportunity Available 57A

Medical Center of Delaware
Opportunity Available 57A

Medical East
Opportunity Available 58A

Medical West
Opportunity Available 56A

Merck Sharp & Dohme
Mefoxin 44A,45A,46A

Michael Reese Health Plan
Opportunity Available 56A

Mount Sinai Medical Center of New York, The
Opportunity Available 51A

Mount Sinai Services
Opportunity Available 56A

Northwestern University Medical School
Opportunity Available 57A

Ortho Pharmaceutical Corporation
All-Flex 4A
Ortho-Novum 7/7/7 ..... Third Cover,Fourth Cover

Osler Institute
Course 66A

Perinatal Associates of Northern California
Opportunity Available 56A

Permanente Medical Group, The
Opportunity Available 51A,53A,55A,59A

Permanente Medical Group Northern California
Opportunity Available 10A

Planned Parenthood of Tomkins County
Opportunity Available 54A

Rochester Medical Group, PC
Opportunity Available 55A

Southwest Medical Associates
Opportunity Available 55A

St. Luke's Hospital
Opportunity Available 54A

St. Vincent's Medical Center
Opportunity Available 54A

Stuart Pharmaceuticals
Cefotan 7A,8A

Syntex Laboratories, Inc.
Tri-Norinyl ..... Second Cover, 1A,2A

Temple University
Opportunity Available 60A

Union Memorial Hospital, The
Opportunity Available 60A

United States Air Force
Recruitment 13A

University of Dundee
Opportunity Available 60A

University of Illinois College of Medicine, The
Opportunity Available 52A,60A

University of Kansas Medical Center
Opportunity Available 61A

University of Missouri-Columbia
Opportunity Available 61A

University of South Dakota School of Medicine
Opportunity Available 61A

University of Texas Medical Branch
Opportunity Available 61A

University of Utah
Opportunity Available 62A

Western Medical Center
Opportunity Available 62A

Worthington Medical Center
Opportunity Available 63A

Wright State University School of Medicine
Opportunity Available 50A

Wyeth-Ayerst Laboratories
Premarin 20A,21A,22A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

The Osler Institute
Obstetrics and Gynecology Review Course
November 26-December 1, 1990 – Chicago
February 2-7, 1991 – Los Angeles
Now, your course with case-list reviews and mock oral exams
Sponsored by the Osler Institute and Penn State

OBJECTIVES
- Increase basic knowledge and clinical skills in ob-gyn.
- Assist residents and fellows to organize study
- Prepare exam candidates to pass their tests.
- Provide practicing gynecologists with an update

METHODS
- HOME STUDY MATERIALS with questions and answers
- SEMINAR with projection slides and syllabus
- LABORATORY with microscopic slides
- PRACTICE EXAMS with oral and written parts

*"The faculty was outstanding. The most pleasant thing was learning a tremendous amount, not only from world-famous authorities but from people who are relatively unknown as well."**

TOPICS

Clinical Science
- Surgical Anatomy
- Embryology
- Radiology
- Genetics
- Teratology
- Anesthesiology
- Antibiotics

Pathology
- Vulva
- Vagina
- Cervix
- Endometrium
- Myometrium
- Ovary
- Placenta

Reproductive Endocrinology
- Contraception
- Infertility
- Amenorrhea
- Abnormal Bleeding
- Androgen
- Prolactin
- Menopause

Oncology
- Chemotherapy
- Vulva and Vagina
- Cervix
- Uterus
- Ovary
- Trophoblast
- Breast

Benign Gynecology
- Infectious Diseases
- Urinary Retention
- Urinary Incontinence
- Pediatric Gynecology
- Sexual Assault
- Ectopic Pregnancy
- Endometriosis

Maternal and Fetal Medicine
- Pharmacology
- Diabetes
- Hypertension
- Hematology
- Antepartum Testing
- Premature Labor
- Birth Trauma

FACULTY

Peter Cherouny, M.D.
Penn State University

Phillip Galle, M.D.
Southern Illinois University

Edward Hill, M.D.
Univ. of Calif., San Francisco

Karen Ireland, M.D.
Oregon Health Science Univ.

Allan Jacobs, M.D.
St. Louis University

Conley Lacey, M.D.
Scripps Clinic, La Jolla, Calif.

David Plourd, M.D.
Santa Clara Valley Med. Ctr.

Michael Policar, M.D.
Univ. of Calif., San Francisco

Gerald Quirk, M.D.
University of Arkansas

Robert Scully, M.D.
Harvard University

Ramona Slupik, M.D.
Northwestern University

Marion Verp, M.D.
University of Chicago

Richard Voet, M.D.
Univ. of Texas, Southwestern

Wendell Wall, M.D.
University of North Dakota

Stan Williams, M.D.
University of Florida

*"Accommodations were comfortable...."**

GOALS AND LOCATION: This course for residents and practicing gynecologists is a comprehensive review and update offered in the spring and fall. The fall course is more clinically oriented. Home study questions are sent upon registration. Your best value is to repeat the course at half price immediately before exams. The November course will be at the Hilton Inn, 3003 Corporate West Drive, Lisle, Illinois. Rates will be $70 single – $90 double.

*"...and those little extras...."**

LOWEST AIR FARES: Please call toll-free 1-800-548-8185 for group discounts.

*"...the most education for the money."**

CATEGORY 1 C.M.E. AND FEES:
- Penn State designates this continuing medical education activity for **60 credit hours in Category 1** of the Physician's Recognition Award of the American Medical Association.
- This program has been approved for **60 cognates, Formal Learning,** by The American College of Obstetricians and Gynecologists.
- Practicing Gynecologists: $720
- Residents and Fellows: $480
- Repeating course within 3 years: $360
- Case-list reviews and mock oral exams (half hour sessions) $ 70
- Optional extra day Dec. 2 (more questions, answers and pathology lab): $150
- **Add 10% for within 10 days of the course.**
- Attendees not in course hotel add $20/day.
- A deposit of $50 will reserve your position.

*"...home study material was extremely helpful."**

CANCELLATIONS: Refunds subject to $50 fee, will be made until the seminar begins.

*"...remarkably complete and pleasant."**.

INFORMATION:
Joseph H. Selliken, Jr., M.D.
The Osler Institute
1094 Dawn Lane, P.O. Box 2218
Terre Haute, IN 47802
(800) 356-7537 or (812) 299-5658

* *Comments by participants*.

Limited Enrollment: **Obstetrics and Gynecology Review Registration**

Name _____

Address _____

City/State/Zip _____

Phone _____

Mail today to:

1094 Dawn Lane, Dept. 11
P.O. Box 2218
Terre Haute, IN 47802

For: ☐ Nov. 26-Dec. 1, 1990 – Chicago
☐ February 2-7, 1991 – Los Angeles
☐ Check enclosed for $_____
☐ Please send more information

ORTHO-NOVUM® 7/7/7 Tablets

Spectacular!



norethindrone/ethinyl estradiol

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28 day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

ORTHO PHARMACEUTICAL CORPORATION
Raritan, NJ 08869-0602

ORTHO



†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, a brief summary of which appears on the preceding page.

© OPC 1989
OJ-709R

Low breakthrough bleeding†

Low-impact norethindrone—no significant change in mean values for total cholesterol, HDL, and LDL

For OC patients of all ages

21- and 28-day regimen

ORTHO-NOVUM® 777

norethindrone/ethinyl estradiol

Tablets

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

16.4.91

November 1990
in two parts, part 2
volume 163, *number* 5

SUPPLEMENT TO

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

PULSATILE GnRH IN CLINICAL MEDICINE

Fort Myers, Florida
January 20, 1990

Co-chairs
Robert B. Jaffe, MD
Samuel S. C. Yen, MD, DSc

Co-sponsored by the Department of Obstetrics, Gynecology and Reproductive Sciences and Extended Programs in Medical Education, University of California School of Medicine, San Francisco

Supported by an educational grant from
ORTHO PHARMACEUTICAL CORPORATION



Published by
MOSBY-YEAR BOOK, INC.
St. Louis, Missouri 63146-3318

ISSN 0002-9378

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

PULSATILE GnRH IN CLINICAL MEDICINE

Fort Myers, Florida
January 20, 1990

Co-chairs

Robert B. Jaffe, MD
Director of Reproductive Endocrinology Center
Professor and Chairman of Department of Obstetrics, Gynecology and Reproductive Sciences
University of California, San Francisco
San Francisco, California

Samuel S. C. Yen, MD, DSc
W. R. Persons Professor of Reproductive Medicine
Director of Reproductive Endocrinology
University of California, San Diego
La Jolla, California

Co-sponsored by the Department of Obstetrics, Gynecology and Reproductive Sciences and Extended Programs in Medical Education, University of California School of Medicine, San Francisco

Supported by an educational grant from
ORTHO PHARMACEUTICAL CORPORATION

Mosby
Year Book

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1990 by Mosby–Year Book, Inc.

Contents

November
Part 2
1990

Introductory remarks 1719
Robert B. Jaffe, MD and Samuel S. C. Yen, MD, DSc
San Francisco and La Jolla, California

The GnRH pulse generator 1721
Ernst Knobil
Houston, Texas

The GnRH pulse generator is located in the arcuate nucleus and its release of GnRH is caused by electrical activity in this area.

Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women 1727
Robert B. Jaffe, MD, Shayne Plosker, MD, Lorna Marshall, MD, and Mary C. Martin, MD
San Francisco, California

A complex, interdependent, multifaceted interaction of steroids, opioids, and catecholamines exists in the regulation of gonadotropin-releasing hormone in women.

Hypothalamic amenorrhea: Clinical perspectives, pathophysiology, and management 1732
James H. Liu, MD
Cincinnati, Ohio

Development of functional hypothalamic amenorrhea reflects a response to environmental stressors and life-style variables, and the underlying defect is a decrease in hypothalamic gonadotropin-releasing hormone secretion.

Vol. 163, No. 5, Part 2, November 1990. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send change of address to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 4351. Annual subscription rates for 1991: domestic, $86.00 for individuals and $150.00 for institutions. Printed in the U.S.A.
Copyright © 1990 by Mosby–Year Book, Inc.

Contents

Polycystic ovary syndrome: Abnormalities and management with pulsatile gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs 1737

Marco Filicori, MD, Carlo Flamigni, MD, Elisabetta Campaniello, MD, Maria Cristina Meriggiola, MD, Laura Michelacci, MD, Alessandro Valdiserri, MD, and Paola Ferrari, BSc

Bologna, Italy

Pretreatment with gonadotropin-releasing hormone analogs immediately followed by pulsatile gonadotropin-releasing hormone achieves high ovulatory and pregnancy rates in polycystic ovarian disease.

The luteinizing hormone–releasing hormone pulse generator in men: Abnormalities and clinical management 1743

Alvin M. Matsumoto, MD, Kenneth M. Gross, MD, Carol B. Sheckter, MD, and William J. Bremner, MD, PhD

Seattle, Washington

Pulsatile luteinizing hormone–releasing hormone treatment is effective in restoring testicular function in men with hypogonadotropic eunuchoidism and decreasing serum follicle-stimulating hormone levels in men with idiopathic oligoazoospermia.

Gonadotropin-releasing hormone deficiency in men: Diagnosis and treatment with exogenous gonadotropin-releasing hormone 1752

William F. Crowley, MD, and Randall W. Whitcomb, MD

Boston, Massachusetts

Treatment of idiopathic hypogonadotropic hypogonadism by administration of exogenous gonadotropin-releasing hormone stimulates puberty and develops normal virilization.

(Contents continued on page 4A)

1991 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)* Canada	Mexico	Region 1	Region 2	Region 3
Institutions†	$150.00	$182.00	$215.00	$223.00	$238.25	$290.50	$342.50
Individuals‡	$ 86.00	$118.00	$181.00	$159.00	$174.25	$226.50	$278.50
Students, residents‡	$ 40.00	$ 72.00	$105.00	$113.00	$128.25	$180.50	$232.50

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

*Airmail breakdown—*Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Contents continued from page 3A

Efficacy and safety of intravenous pulsatile gonadotropin-releasing hormone: Lutrepulse for Injection 1759
Nanette Santoro, MD
Newark, New Jersey

Pulsatile gonadotropin-releasing hormone effectively restored physiologic ovarian function in a large group of women with hypothalamic amenorrhea, entailing very few side effects.

Practical aspects of pulsatile gonadotropin-releasing hormone administration 1765
Eldon D. Schriock, MD
Memphis, Tennessee

Pulsatile gonadotropin-releasing hormone therapy is a safe, effective, convenient, and economic alternative to human menopausal gonadotropin for ovulation induction in women resistant to clomiphene.

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 163 *number* 5 *part* 2 NOVEMBER 1990

Introductory remarks

Robert B. Jaffe, MD, and Samuel S. C. Yen, MD, DSc

San Francisco and La Jolla, California

In recent years, there has been a remarkable expansion of our understanding of endocrinologic events and in the translation of this basic understanding into the clinical arena. A seminal observation in reproductive neuroendocrinology was made in 1970 by Knobil et al.,[1] who reported on the oscillatory fluctuations in luteinizing hormone (LH) concentration in the plasma of ovariectomized monkeys. Now, 20 years later, this observation has led to the introduction of pulsatile gonadotropin-releasing hormone (GnRH) and to the development of agonistic and antagonistic analogs of GnRH to treat a variety of endocrinologic disorders.

During the intervening 20 years, many of the complex mechanisms involved in the neuroendocrinologic control of gonadotropin secretion were gradually and elegantly revealed. The physiologic importance of the pulsatile nature of gonadotropin secretion was demonstrated in 1978, when Belchetz et al.[2] reported that only pulsatile and not continuous administration of exogenous GnRH could reestablish gonadotropin secretion in monkeys deprived of endogenous GnRH by radiofrequency lesioning the arcuate nucleus of the hypothalamus, the site of most GnRH production. Moreover, the frequency of pulsatile GnRH administration was critical. In monkeys the ideal frequency was 60 minutes[3]; however, recent studies in humans have shown that frequencies of 60 to 120 minutes are effective in restoring normal gonadotropin secretory patterns.

Most of the early investigations into the neuroendocrinology of gonadotropin secretion and regulation were carried out in experimental animal systems. Nature, however, has provided its own set of experimental models. The disorders of gonadotropin secretion for which pulsatile GnRH may provide an ideal treatment, such as hypothalamic amenorrhea, also provide investigators with ideal systems in which to study the regulatory mechanisms involved in GnRH function.

Hypothalamic amenorrhea can result from a number of physiologic and environmental conditions. However, the common features in persons with this disorder are intact pituitary-ovarian-endometrial function and a deficiency in the pulsatile secretion of GnRH. The neuroendocrinologic basis for this deficiency is not definitely known; these women provide a useful model for the study of the regulatory mechanisms involved in normal gonadotropic function. From a clinical standpoint, these women are anovulatory, and many of them desire pregnancy. Because the pituitary-ovarian axis is operative in these women, GnRH, when presented in a physiologic, pulsatile manner, can be quite effective in inducing ovulation and ultimately pregnancy.

Although the cause of polycystic ovary syndrome is quite distinct from that of hypothalamic amenorrhea, the clinical concerns may be similar. Anovulation is common in these women, and ovulation induction is often necessary to treat infertility. Since existing methods of ovulation induction are frequently ineffective, more effective means of induction would be desirable. Pulsatile GnRH alone has not been very effective in inducing ovulation in this disorder. However, the use of a GnRH analog to create a hypogonadotropic state before the administration of pulsatile GnRH shows great promise as a treatment for anovulation in women with polycystic ovary syndrome.

Disorders of GnRH pulse generation have also been identified in men. In idiopathic hypogonadotropic hypogonadism, LH secretion is usually apulsatile, an observation that suggests the absence or severe reduction of GnRH pulse generation. Treatment of idiopathic hypogonadotropic hypogonadism with testosterone therapy is usually sufficient to promote pubertal development and normal sexual function. In patients in whom induction of sperm production and fertility are desired, long-term therapy with pulsatile GnRH has proved quite successful in experimental studies. With

From the Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, and the Department of Reproductive Medicine, University of California, San Diego.

Reprint requests: Robert B. Jaffe, MD, Director, Reproductive Endocrinology Center, University of California, San Francisco, 505 Parnassus, San Francisco, CA 94143.

6/0/24912

the availability of GnRH for clinical use, replacement therapy for men with idiopathic hypogonadotropic hypogonadism should now become a real option.

This symposium integrates the basic research into the physiologic principles of GnRH pulse generation and the clinical applications to which this basic research has so logically led. The clinical disorders that will be discussed result from a defect in the pulsatile secretion of GnRH by the hypothalamus. The use of pulsatile GnRH in the treatment of these disorders is inherently attractive because it is a physiologic means of correcting a physiologic defect.

REFERENCES

1. Dierschke DJ, Bhattacharya AN, Atkinson LE, Knobil E. Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey. Endocrinology 1970;87:850-3.
2. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science 1978;202:631-3.
3. Nakai Y, Plant TM, Hess DL, Keogh EJ, Knobil E. On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. Endocrinology 1978;102:1008-14.

The GnRH pulse generator*,**

Ernst Knobil

Key words: Gonadotropin-releasing hormone, multiunit electrical activity, luteinizing hormone, arcuate nucleus

The notion of an oscillator or signal generator in the central nervous system that controls the rhythmic release of GnRH and, thereby, the pulsatile secretion of the gonadotropic hormones, originated in the finding of strikingly abrupt and rhythmic fluctuations in the concentration of LH in the plasma of ovariectomized monkeys. These oscillations had a period of about one hour when blood samples were obtained at 10 to 20 minute intervals.[1]

These surprising observations were presaged by reports of seemingly random, major fluctuations in plasma gonadotropin concentrations in gonadectomized monkeys[2] and rats[3, 4] as well as in women.[5] In these earlier studies the sampling intervals employed were never less than one hour and unable, therefore, to reveal the orderly, rhythmic events that occur with frequencies of one event per hour, or more.

In the original study describing pulsatile LH secretion in ovariectomized monkeys the possibility was considered that the circhoral discharges of LH may be initiated by autoregulatory mechanisms involving long or short feedback loops, but the more likely view that these discharges were due to intermittent signals from the central nervous system unrelated to circulating LH levels that resulted in putative increments in GnRH release was favored. Nevertheless, the possible role of autoregulatory mechanisms in the control of pulsatile LH secretion was repeatedly considered,[6] but finally laid to rest in the relatively recent past.[7] Conversely, the view that each pulse of LH released from the pituitary gland is the consequence of a bolus of GnRH secreted into the pituitary portal system has been unequivocally upheld by the demonstration of synchronous increments of GnRH assessed in the pituitary portal circulation and of LH measured in samples of peripheral blood obtained simultaneously.[8, 9] In any case, the phenomenon of pulsatile gonadotropic hormone secretion was rapidly extended to most vertebrate species studied in this regard, including our own, and forms the basis of an increasingly voluminous, rapidly expanding literature.

In the monkey[10] as in the rat[11] total surgical deafferentation of the mediobasal hypothalamus does not interfere with pulsatile gonadotropic hormone secretion suggesting that what has become known as the GnRH pulse generator lies within this structure. Somewhat more precise localization was achieved in the rhesus monkey by the demonstration that bilateral destruction of the area comprising the arcuate nuclei promptly abolished gonadotropin secretion without notable effects on the production of the other adeno- and neurohypophysial hormones.[12] That the GnRH pulse generator is an intrinsic property of the medial basal hypothalamus has been affirmed by the demonstration of pulsatile GnRH release at appropriate frequencies from superfused hypothalamic fragments in vitro.[13]

The physiologic significance of the pulsatile mode of gonadotropin secretion became apparent with the finding that gonadotropin secretion could be reestablished in rhesus monkeys deprived of endogenous GnRH secretion by destruction of the arcuate nucluei only by the intermittent administration of exogenous GnRH at the physiologic frequency of one pulse per hour, continuous infusion of the decapeptide provoking but an evanescent response followed by desensitization of the gonadotrophs.[14, 15] In fact, the administration of GnRH at an unvarying frequency of one pulse per hour to such animals supported normal, ovulatory menstrual cycles affirming the conclusion[14] that, in this species, hypothalamic GnRH secretion is a permissive component of the ovarian cycle rather than its controller.[16] More to the point, the pulsatile administration of GnRH to prepubertal female monkeys promptly established normal ovarian function including 28-day ovulatory menstrual cycles.[17] These findings demonstrated that nothing below the hypothalamus was limiting in the advent of puberty and suggested that the pubertal process may be initiated by an activation of the hypothalamic GnRH pulse generator. This fundamental issue is discussed at length by other contributors to this symposium and will not be reconsidered here.

The central importance of the GnRH pulse generator as a limiting component of the control system that governs reproductive processes has been widely documented in a number of species including man (see 18, 19, 20, 21 for review). Much of what is known about

Laboratory for Neuroendocrinology, The University of Texas Health Science Center, Medical School, Houston, Texas USA.

**Reprinted from a contribution to the Control of the Onset of Puberty III, eds. H. A. Delemarre-van de Waal, T. M. Plant, G. P. van Rees and J. Schoemaker. Elsevier Science Publishers B. V. (Biomedical Division) Amsterdam, 1989.*

***This account is not intended to be a comprehensive review. Rather it represents the author's somewhat myopic view of the subject based on his intimate association with it for the past 20 years.*

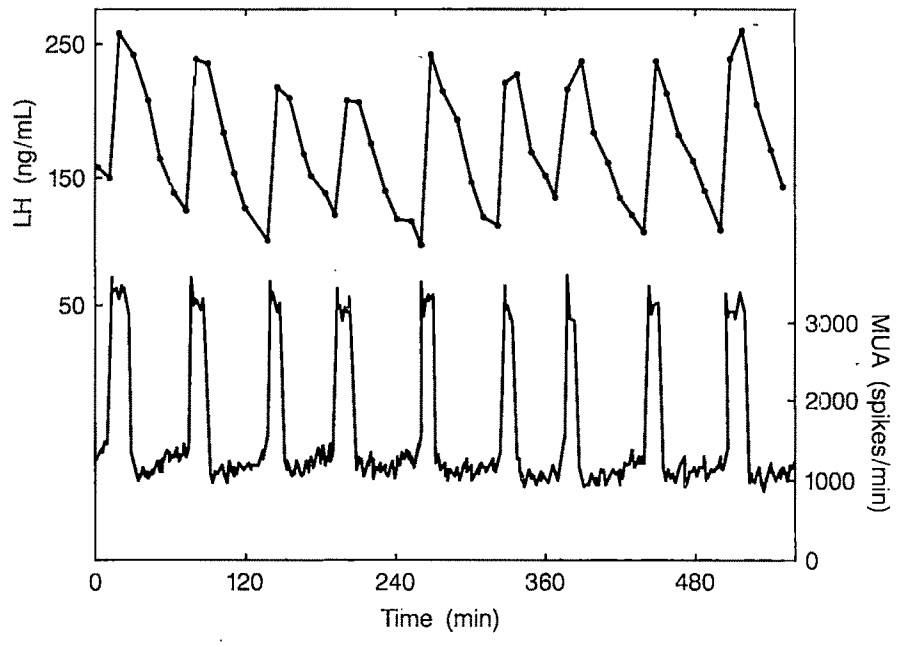


Fig. 1. Characteristic volleys of electrical activity *(MUA)* recorded from the medial basal hypothalamus in relation to LH pulses in the peripheral circulation in an ovariectomized monkey.

the operation of this neuronal system is based on the monitoring of LH pulses in the peripheral circulation. While such pulses may provide an unambiguous reflection of the pulsatile discharges of GnRH into the pituitary portal circulation in some circumstances, notably when negative feedback loops are open and experimental influences directly on pituitary function have been eliminated, this approach has clear limitations. In the presence of the gonads, the magnitude of the LH pulses is much reduced and requires rigorous statistical techniques for their identification[22] and even these do not always eliminate uncertainty. More importantly, the operation of the pulse generator cannot be monitored by this approach in the complete absence of LH pulses attributable to blockade of LH release at the pituitary level.

While, at the moment, there are no alternatives to this approach in human subjects, more direct assessments of GnRH pulse generator activity have been attempted in experimental animal preparations. In addition to the measurement of GnRH in pituitary portal blood and in the superfusion medium from isolated hypothalamic preparations *in vitro* already mentioned, these have included the measurement of GnRH in hypothalamic extracellular fluid using the "push-pull" technique[23, 24, 25] or in cerebrospinal fluid sampled from the third ventricle.[26]

Over a decade ago our own laboratory initiated an alternative, direct approach to the GnRH pulse generator based on the supposition that the discharge of GnRH from the terminals of GnRH cells must be a consequence of action potentials as demonstrated in a variety of other neurosecretory systems. We further reasoned that the intermittent release of GnRH in physiologically effective quantities must reflect the simultaneous discharge of a number of GnRH cells and that the antecedent, synchronous electrical events should be measurable using multiunit recording techniques as demonstrated for the magnocellular component of the hypothalamus in the rhesus monkey and other species. We thus applied the techniques devised by Hayward and his associates[27] for measuring the multiunit electrical activity (MUA) generated by vasopressin cells responding to appropriate osmotic stimuli to the search for the GnRH pulse generator of the rhesus monkey. Our strategy was based on the fact that we knew where to place the recording electrodes (in the region of the arcuate nucleus) and on the availability of unambiguous points of reference provided by the circhoral LH pulses seen in ovariectomized animals. While early results[28] were encouraging and prompted us to continue the search, successful outcomes occurring with acceptable frequency were not obtained until we switched from single, acutely placed electrodes to permanently implanted bilateral arrays of multiple electrodes that essentially blanketed the entire medial basal hypothalamus.[29] Dramatic increments in MUA coincident with the initiation of LH pulses, as measured in the periphery, could then be demonstrated with reasonable reproducibility (Figure 1). Because barbiturate anesthesia does not abolish the pulsatile secretion of LH in the rhesus monkey,[30] such results were initially obtained in animals anesthetized with pentobarbital to reduce electrical noise associated with muscular movement and

to minimize unrelated central electrical activity. It was found subsequently that the MUA discharges associated with LH pulses ("volleys" of electrical activity, as we have come to call them) were demonstrable with equal clarity in awake ovariectomized monkeys restrained in primate chairs.[29]

These MUA volleys were so striking that we initially suspected them to be artifactual despite their invariable coincidence with LH pulses, but only because we had expected, when we began the attempt, that the likelihood of success was vanishingly small and that, if we were to see anything at all, it would be subtle and difficult to discern without the assistance of statistical manipulation designed to distinguish pulse generator signals from ambient neuronal electrical activity. We were eventually reassured that we were indeed monitoring the activity of the GnRH pulse generator by the outcome of a series of pharmacological interventions previously shown to modify the frequency of LH pulses in the ovariectomized rhesus monkey (see below). In all of them, every alteration in LH pulse frequency was mirrored in simultaneous changes in MUA volleys. To date, in literally thousands of observations, every MUA volley observed has been followed by an LH pulse. The converse, of course, does not obtain. In animals that have regular LH pulses but no concomitant changes in electrical activity, the electrodes simply have missed their mark and electrode reimplantation usually yields a useful preparation.

In general, the hypothalamic structures from which the successful recordings have been made grossly coincide with the GnRH neuronal system of the rhesus monkey previously identified by immunocytochemical techniques.[31] Whether the recordings are made from GnRH cells or fibers or from cells or fibers that do not contain GnRH could not be ascertained because of the relatively large diameter (50 μm) of the recording electrodes relative to these structures.[31]

The pharmacological studies already alluded to, beyond validating the specificity of our electrophysiological observations, have also provided some insight relevant to the operation of the GnRH pulse generator but not sufficiently so to permit the construction of compelling models. Barbiturates slow the frequency of the system in dose-dependent fashion[29, 32] as does prolonged treatment with ketamine. Alpha-adrenergic and dopaminergic blockade also reduces the frequency of MUA volleys and of the consequent LH pulses.[33] Similarily, morphine dramatically interrupts the firing of the pulse generator, an action blocked by naloxone, while naloxone alone, at the doses given was without effect in these ovariectomized animals,[34] suggesting minimal opiate tone in the absence of the gonads.

Because *N*-methyl D-aspartic acid (NMDA) is a potent stimulator of the LH release in the rhesus monkey[35, 36] the role of NMDA receptors in the operation of the GnRH pulse generator was investigated. Our preliminary findings to date indicate that NMDA administration to ovariectomized monkeys indeed elicits MUA volleys followed by LH pulses. NMDA receptor blockade with AP-5, administered intracerebrally, appears to have a range of dose-independent effects on pulse generator activity, from prolonged arrest to minor delays of the MUA volleys that immediately follow the injection of the drug.[37] The most dramatic effects of the blocker were associated with profound behavioral disturbances and could, therefore, have been due to non-specific actions of the drug. The non-competitive inhibitor MK 801, while inducing anesthesia was, in a single animal, without effect on pulse generator activity.[37]

Because the frequency of LH pulses (circhoral) has been reported to be essentially the same during the follicular phase of the menstrual cycle, when estrogen levels are maximal, and in the absence of ovarian function, when they are immeasurable, it was concluded that estradiol in physiological concentrations does not have a significant effect on pulse generator frequency.[21] We were surprised to find, therefore, that physiological estrogen replacement in ovariectomized monkeys profoundly inhibited pulse generator activity.[38] The reason for this difference between intact and ovariectomized monkeys remains to be resolved but the finding is reminiscent of earlier observations showing remarkable differences in the apparent steroidal control of gonadotropin secretion between ovariectomized and normal animals.[39] It was noted in passing that the reduction in pulse generator frequency in ovariectomized monkeys occasioned by physiological levels of estradiol was accompanied by a decrease in the *duration* of individual MUA volleys.[38] The control of volley duration, in contradistinction to pulse generator frequency, and its significance are discussed further below.

Our purpose from the outset of our electrophysiological efforts was to study the function of the GnRH pulse generator throughout the menstrual cycle of the rhesus monkey and in other physiological settings such as the initiation of puberty. Such investigations, obviously, can only be conducted in intact animals. While all of the foregoing work in ovariectomized monkeys was being conducted, attempts to study the pulse generator in normal animals were unrelentingly pursued but without success. In the original observations of LH pulses in ovariectomized monkeys it was noted that these could not be seen in intact females.[1] It was supposed at the time, and not entirely incorrectly in retrospect, that in the presence of the ovaries and a closed negative feedback loop, the LH pulses were too small to be detected by our radioimmunoassay. It was discovered nearly 20 years later, however, when an ex-

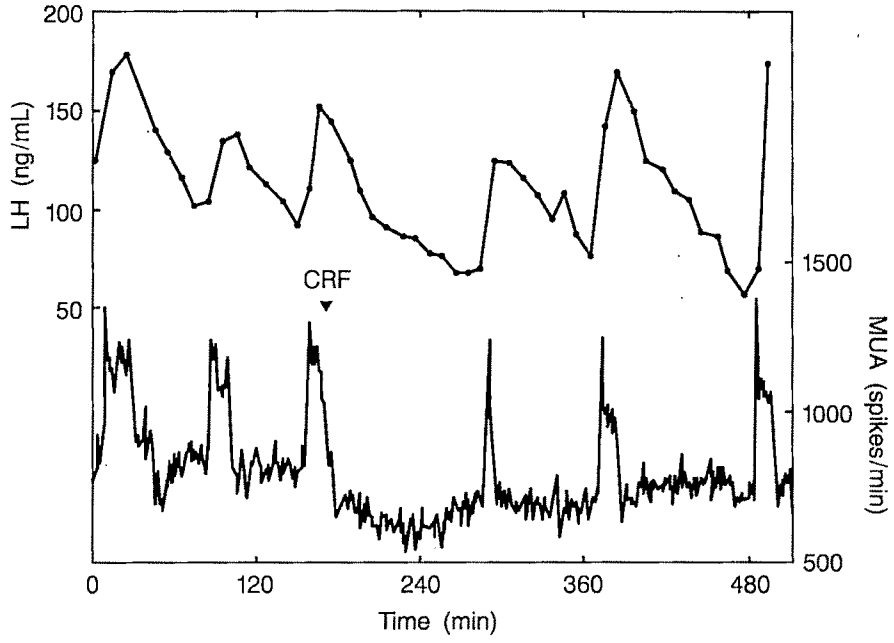


Fig. 2. Effect of a single intravenous injection of corticotropin-releasing factor *(CRF)*, at *arrow*, on GnRH pulse generator activity in an ovariectomized rhesus monkey.[12]

tremely sensitive bioassay was substituted for the immunoassay, that LH pulses still could not be detected in normal monkeys restrained in primate chairs. We gradually came to the realization that the mild "stress" of chair restraint, to which the animals appeared to be accustomed, had arrested the GnRH pulse generator. Blood samples taken from intact animals under barbiturate anesthesia or otherwise tranquilized did show clear LH pulses, as well as the attendant MUA volleys, albeit at a reduced frequency. Michel Ferin and his colleagues had also noted that chair restraint interferes with pulsatile LH secretion in intact monkeys and that this could not be reversed by naloxone administration.[10] We have found, as they did, that corticotropin releasing hormone (CRH) inhibits pulsatile LH secretion, and that the effect of the neuropeptide could occasionally be reversed by naloxone[11, 12] but the relationship between these observations and the consequences of stress remain to be clarified. An example of this phenomenon is shown in Figure 2. In any case, the remarkable difference between ovariectomized and intact monkeys in the resistence of the GnRH pulse generator to stress presents a problem of fundamental importance.

Intact rhesus monkeys, fitted with recording electrodes and chronic indwelling cardiac catheters do exhibit normal, ovulatory menstrual cycles and must, therefore have a normally functioning hypothalamic GnRH pulse generator when they are not subject to the brief periods of restraint in primate chairs. Although pulse generator activity in such animals was easily demonstrable when CNS depressants were given, thus bypassing chair restraint, this obviously did not provide a satisfactory approach to the study of normal hypothalamic function and an alternative strategy had to be devised. For this purpose, we developed a radiotelemetry system composed of a radiotransmitter to which the distal end of a recording electrode is attached, a receiving antenna placed in close proximity to a suitably modified cage and an ordinary FM receiver.[13] The hypothalamic signals are then processed in a manner identical to the "hard wired" system previously described.[29] This technique permits continuous recording of MUA activity in unrestrained animals for unlimited periods of time. More recently, we have found that intensive habituation of normal monkeys to primate chair restraint, for extended periods, reestablishes normal GnRH pulse generator activity and provides the opportunity to study the system in the presence of the ovaries, at least for the limited time offered by a single experimental day, the maximum period of restraint that we have employed.

These rather arduous methodologies, developed over a period of years, are only now beginning to provide reliable descriptions of the function of the GnRH pulse generator in the normal rhesus monkey. Among these is the confirmation of earlier findings in the human and monkey (see 21 for review) that pulse generator frequency is markedly reduced during the luteal phase of the cycle as well as during the night, especially during the follicular phase of the cycle, suggesting an interaction between circadian timing mechanisms and the circhoral signal generator of the hypothalamus.

Totally unexpected, however, was the finding, to be published in detail elsewhere, of an abrupt reduction

in the frequency of the pulse generator coincident with the preovulatory, mid-cycle LH surge. Again, this is accompanied by concurrent reductions in LH pulse frequency.

Another unanticipated observation was the marked difference in the duration of MUA volleys between normal and ovariectomized animals. The period of increased electrical activity of the GnRH pulse generator lasts for five minutes, on the average, in normal monkeys during the follicular phase of the cycle in contrast to about 20 minutes in ovariectomized animals.[14] As already mentioned, estradiol administered to ovariectomized animals seemed to shorten the duration of MUA volleys as well as reduce the frequency of the pulse generator. In a more recent study it has been found that while naloxone blocks the effect of estradiol on pulse generator frequency, it has no effect on the action of the steroid on MUA volley duration. Morphine, however, mimics the actions of estradiol, but when administered at an appropriate rate, can reduce volley duration without significant effect on frequency.[14] Taken together, these observations suggest that frequency and duration of activation of the GnRH pulse generator can be controlled independently. Because the duration of MUA volleys is unrelated to the amount of LH released per pulse it appears that the full complement of GnRH released by each activation of the pulse generator is discharged at the onset of electrical activity.[15] While control of MUA volley duration may be of little moment in terms of LH secretion, it may provide potentially important insights regarding the functioning of the pulse generator at the cellular level.

Despite the contemplation of the GnRH pulse generator in terms of its outputs for two decades, the relentless probing of its workings with pharmacological tools and the deciphering of its electrophysiological substrate, we remain woefully ignorant of the neuronal construct that underlies its activity. That the pulse generator is an intrinsic property of the MBH, probably of the arcuate nucleus, seems reasonably certain. In the monkey and human it has a frequency of approximately one pulse per hour in its unmodulated (open loop) state and this frequency is maximal or close to it. The elementary question of whether the pulse generator resides in GnRH neurons or in cells that eventually synapse with them remains to be definitively answered.

The simplest hypothesis is that a single pacemaker cell, or small group of cells, initiates each pulse and entrains all other participants in the consequent neuroendocrine cascade, thus accounting for the mandatory synchrony of GnRH release. But the circhoral frequency of such pacemaker neurons, if they exist, is difficult to explain in terms of currently understood neurocellular control systems. The finding, however, that the pacemaker for the circadian rhythm of the marine snail *Bulla* resides in a small cluster of cells at the base of its eyestalks, and that only one of these cells may be capable of timing the entire circadian behavior pattern of this animal[16] provides a small measure of credibility to the possibility that the pacemaker for a circhoral rhythm may also reside in kindred cellular domains. But as is often the case, the simplest models are unequal to the task and more complex hypotheses may eventually have to be tested and affirmed or rejected in their turn.

Acknowledgements

The work of the Author's laboratory is being supported by Grants HD17438 and HD08610 from the National Institutes of Health and by the Clayton Foundation for Research.

REFERENCES

1. Dierschke DJ, Bhattacharya AN, Atkinson LE, Knobil E. Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey. Endocrinology 1970;87:850.
2. Atkinson LE, Bhattacharya AN, Monroe SE, Dierschke DJ, Knobil E. Effects of gonadectomy on plasma LH concentration in the rhesus monkey. Endocrinology 1970; 87:847.
3. Lawton IE, Schwartz NB. A circadian rhythm of luteinizing hormone secretion in ovariectomized rats. Am J Physiol 1968;214:213.
4. Gay VL, Midgley AR. Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay. Endocrinology 1969;84:1359.
5. Dignam WJ, Parlow AF, Daane TA. Serum FSH and LH measurements in the evaluation of menstrual disorders. Am J Obstet Gynecol 1969;105:679.
6. Knobil E. On the control of gonadotropin secretion in the rhesus monkey. Rec Prog Horm Res 1974;30:1.
7. Kesner JS, Kaufman JM, Wilson RC, Kuroda G, Knobil E. On the short-loop feedback regulation of the hypothalamic luteinizing hormone releasing hormone "pulse generator" in the rhesus monkey. Neuroendocrinology 1986;42:109.
8. Clarke IJ, Cummins JT. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology 1982;111:1737.
9. Moenter SM, Woodfill CJI, Caraty A, Karsch FJ. The estradiol induced GnRH surge in the ewe. [Abstract]. Biol Reprod 1989;40(suppl 1):354.
10. Krey LC, Butler WR, Knobil E. Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. 1. Gonadotropin secretion. Endocrinology 1974;96:1073.
11. Blake CA, Sawyer CH. Effects of hypothalamic deafferentation on the pulsatile rhythm in plasma concentrations of luteinizing hormone in ovariectomized rats. Endocrinology 1974;94:730.
12. Plant TM, Krey LC, Moossy J, McCormack JT, Hess DL, Knobil E. The arcuate nucleus and the control of gonadotropin and prolactin secretion in the female rhesus monkey *(Macaca mulatta)*. Endocrinology 1978;102:52.
13. Bourguignon JP, Franchimont P. Intermittent liberation of gonadoliberin (GnRH) by the rat hypothalamus in vitro. C R Soc Biol (Paris) 1981;175:389.
14. Nakai Y, Plant TM, Hess DL, Keogh EJ, Knobil E. On

the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. Endocrinology 1978;102:1008.
15. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science 1978;202:631.
16. Knobil E, Plant TM, Wildt L, Belchetz PE, Marshall G. Control of the rhesus monkey menstrual cycle: permissive role of hypothalamic gonadotropin-releasing hormone. Science 1980;207:1371.
17. Wildt L, Marshall G, Knobil E. Experimental induction of puberty in the infantile female rhesus monkey. Science 1980;207:1373.
18. Gallo RV, Babu GN, Bona-Gallo A, Devorshak-Harvey E, Leipheimer RE, Marco J. Regulation of ovarian and testicular function. In: Mahesh VB, Dhindsa DS, Anderson E, Kalra SP, eds. Advances in experimental medicine and biology. New York: Plenum Press, 1987:109.
19. Negro-Vilar A, Culler MD, Valenca MM, Masotto C, Lopez FJ, Ching M. Neural control of pulsatile gonadotropin secretion. In: Serio M, ed. Perspectives in andrology. New York: Raven Press, Serono Symposium Publications, 1989;53:149.
20. Karsch FJ, Bittman EL, Foster DL, Goodman RL, Legan SJ, Robinson JE. Neuroendocrine basis of seasonal reproduction. Rec Prog Horm Res 1984;40:185.
21. Knobil E, Hotchkiss J. The menstrual cycle and its neuroendocrine control. In: Knobil E, Neill JD, eds. The physiology of reproduction. New York: Raven Press, 1988.
22. Veldhuis JD, Evans WS, Johnson ML, Wills MR, Rogol AD. Physiological properties of the luteinizing hormone pulse signal: impact of intensive and extended venous sampling paradigms on its characterization in healthy men and women. J Clin Endocrinol Metab 1986;62:881.
23. Pau KYF, Hess DL, Kaynard AH, Ji WZ, Gliessman PM, Spies HG. Suppression of mediobasal hypothalamic gonadotropin-releasing hormone and plasma luteinizing hormone pulsatile patterns by phentolamine in ovariectomized rhesus macaques. Endocrinology 1989;124: 891.
24. Terasawa E, Krook C, Hei DL, Gearing M, Schultz NJ, Davis GA. Norepinephrine is a possible neurotransmitter stimulating pulsatile release of luteinizing hormone–releasing hormone in the rhesus monkey. Endocrinology 1988;123:1808.
25. Levine JE, Pau KYF, Ramirez VD, Jackson GD. Simultaneous measurement of luteinizing hormone–releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. Endocrinology 1982;111: 1449.
26. VanVugt DA, Diefenbach WD, Alston E, Ferin M. Gonadotropin-releasing hormone pulses in third ventricular cerebrospinal fluid of ovariectomized rhesus monkeys: correlation with luteinizing hormone pulses. Endocrinology 1985;117:1550.
27. Hayward JN. Functional and morphological aspects of hypothalamic neurons. Physiol Rev 1977;57:574.
28. Knobil E. Patterns of hypophysiotropic signals and gonadotropin secretion in the rhesus monkey. Biol Reprod 1981;24:44.
29. Wilson RC, Kesner JS, Kaufman JM, Uemura T, Akema T, Knobil E. Central electrophysiologic correlates of pulsatile luteinizing hormone secretion in the rhesus monkey. Neuroendocrinology 1984;39:256.
30. Bhattacharya AN, Dierschke DJ, Yamaji T, Knobil E. The pharmacologic blockade of the circhoral mode of LH secretion in the ovariectomized rhesus monkey. Endocrinology 1972;90:778.
31. Silverman AJ, Wilson R, Kesner JS, Knobil E. Hypothalamic localization of multiunit electrical activity associated with pulsatile LH release in the rhesus monkey. Neuroendocrinology 1986;44:168.
32. Wehrenberg WB, Ferin M. Alteration of pulsatile LH secretion in monkeys by pentobarbital anesthesia. Proc Soc Exp Biol Med 1981;168:286.
33. Kaufman JM, Kesner JS, Wilson RC, Knobil E. Electrophysiological manifestation of luteinizing hormone–releasing hormone pulse generator activity in the rhesus monkey: influence of alpha-adrenergic and dopaminergic blocking agents. Endocrinology 1985;116:1327.
34. Kesner JS, Kaufman JM, Wilson RC, Kuroda G, Knobil E. The effect of morphine on the electrophysiological activity of the hypothalamic luteinizing hormone–releasing hormone pulse generator in the rhesus monkey. Neuroendocrinology 1986;43:686.
35. Wilson RC, Knobil E. Acute effects of *N*-methyl-DL-aspartate on the release of pituitary gonadotropins and prolactin in the adult female rhesus monkey. Brain Res 1982;248:177.
36. Gay VL, Plant TM. *N*-methyl-D,L-aspartate elicits hypothalamic gonadotropin-releasing hormone release in prepubertal male rhesus monkeys *(Macaca mulatta)*. Endocrinology 1987;120:2289.
37. Grosser PM, Williams CL, Thalabard JC, Nishihara M, Brody S, Knobil E. *N*-methyl-D,L-aspartate (NMDA) receptor blockade of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator activity in the rhesus monkey. Proc Endocrine Soc 1989;Abstract 942.
38. Kesner JS, Wilson RC, Kaufman JM, Hotchkiss J, Chen Y, Yamamoto H, Pardo RR, Knobil E. Unexpected responses of the hypothalamic gonadotropin-releasing hormone "pulse generator": to physiological estradiol inputs in the absence of the ovary. Proc Natl Acad Sci USA 1987; 84:8745.
39. Karsch FJ, Dierschke DJ, Weick RF, Yamaji T, Hotchkiss J, Knobil E. Positive and negative feedback control by estrogen of luteinizing hormone secretion in the rhesus monkey. Endocrinology 1973;92:799.
40. VanVugt DA, Bakst G, Dyrenfurth I, Ferin M. Naloxone stimulation of luteinizing hormone secretion in the female monkey: influence of endocrine and experimental conditions. Endocrinology 1983;113:1858.
41. Gindoff PR, Ferin M. Endogenous opioid peptides modulate the effect of corticotropin-releasing factor on gonadotropin release in the primate. Endocrinology 1987; 121:837.
42. Williams CL, Nishihara M, Hotchkiss J, Thalabard JC, Grosser PM, Knobil E. Site of inhibitory action of corticotropin-releasing factor on gonadotropin secretion in the rhesus monkey. [Abstract]. Proc Endocrine Soc 1988:649.
43. Thalabard JC, Grosser P, Nishihara M, Williams CL, Hotchkiss J, Ladendorf D, Knobil E. Activité electrophysiologique du générateur hypothalamique: approche télémetrique au cours du cycle menstruel chez le singe rhesus. [Abstract]. Proc 8Eme Congr Franc Endocrinol 1988:142.
44. Williams CL, Nishihara M, Thalabard JC, Grosser PM, Hotchkiss J, Knobil E. The hypothalamic GnRH pulse generator of the rhesus monkey: the duration of phasic electrical activity and its control. [Abstract]. Proc Soc Neurosci 1988:431.1.
45. Williams CL, Thalabard JC, Hotchkiss J, Grosser PM, Nishihara M, Knobil E. The duration of phasic electrical activity of the hypothalamic gonadotropin releasing hormone (GnRH) pulse generator and its influence on resultant LH pulse dynamics. [Abstract]. Proc Endocrine Soc 1989:936.
46. Block GD, McMahon DG. Cellular analysis of the bulla

ocular circadian pacemaker system. III. Localization of the circadian pacemaker. J Comp Physiol [A] 1984; 155:387.

Discussion

Audience. In one of your slides there seemed to be a difference at the beginning of the cycle between the follicle-stimulating hormone (FSH) and LH response to pulsatile GnRH. Do you think that the relatively greater increase in FSH than in LH levels is important in folliculogenesis?

Dr. Knobil. At present we do not know the physiologic role of the relative elevation of FSH levels at the beginning of the cycle.

Audience. Have you looked for a similar correlation between electrical spikes and other neuroendocrine hormones or neurotransmitters?

Dr. Knobil. The secretion of all the pituitary hormones, and most peptide neurotransmitters, is episodic. We looked for changes in hormone secretory patterns that are synchronous with LH and FSH, and we could not find any except prolactin, under certain circumstances. It has been found that GnRH, in appropriate circumstances, does stimulate prolactin secretion. So far we have been unable to find electrophysiologic correlates for patterns of peptide hormone secretion other than GnRH.

Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women

Robert B. Jaffe, MD, Shayne Plosker, MD, Lorna Marshall, MD, and Mary C. Martin, MD
San Francisco, California

The pulsatile release of gonadotropin-releasing hormone and the consequent secretion of gonadotropins are regulated by a complex interplay of steroids, neuropeptides, catecholamines, and environmental factors. Estrogen and progesterone influence the amplitude and frequency of luteinizing hormone pulsatile secretion. These effects lead to both a diurnal variation in pulse frequency, with a lower frequency at night, and variation during the menstrual cycle, with a lower frequency and increased amplitude during the luteal phase. Opioid peptides inhibit the pulsatile discharge of gonadotropin-releasing hormone and luteinizing hormone. The opioid antagonist, naloxone, causes an increase in luteinizing hormone secretion, particularly during the luteal phase. The administration of opioid receptor agonists, such as β-endorphin, results in a decline in serum luteinizing hormone during the early follicular phase. Corticotropin-releasing factor, which is increased during stress, inhibits pulsatile luteinizing hormone secretion, and this effect can be blocked by the simultaneous administration of naloxone. These observations suggest that corticotropin-releasing factor exerts its effects on luteinizing hormone through an opioidergic intermediary. Endogenous catecholamines such as dopamine inhibit pulsatile luteinizing hormone release; however, the mechanism involved is not clear. (Am J Obstet Gynecol 1990;163:1727-31.)

Key words: Gonadotropin-releasing hormone, catecholamines, opioids, steroids

Pulsatile discharge of gonadotropins, and thus of gonadotropin-releasing hormone (GnRH), has its origins in intrauterine life. Rasmussen et al.[1] have demonstrated pulsatile release of GnRH from the human fetal pituitary superfused in vitro. In vivo Martin et al.[2] have demonstrated pulsatile release of luteinizing hormone (LH) in the catheterized rhesus monkey fetus in utero. This pulsatile release was evident by at least 130 days' gestation (term = 165 days). The amplitude of LH pulses was greater in female than in male fetuses, presumably because of the restraining action of testicular testosterone in the male. Near term pulsatile LH discharge was markedly diminished in both sexes.

In 1971 Midgley and Jaffe[3] and Yen et al.[4] demonstrated that the episodic release of LH in women varied in amplitude and frequency at different stages of the cycle. Pulses of high frequency and low amplitude were observed during the follicular phase of the cycle, whereas pulses of high amplitude and relatively lower

From the Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco.
Reprint requests: Robert B. Jaffe, MD, Director, Reproductive Endocrinology Center, University of California, San Francisco, 505 Parnassus, San Francisco, CA 94143.
6/0/25004

frequency were observed during the luteal phase. More recently Soules et al.[5] and Marshall et al.[6] demonstrated a nocturnal slowing of LH pulses in women during the follicular phase. Soules et al. did not find this pattern during the late follicular or midluteal phase. All these observations suggest an influence of estrogens and progesterone on the magnitude and frequency of pulsatile LH discharge. Marshall et al. also demonstrated that effecting preovulatory circulating concentrations of estradiol caused a diurnal change in LH pulse discharge. In addition, this concentration of estradiol inhibited LH pulse discharge for the first 3 to 5 hours after sleep. The diurnal pulsatile discharge of LH, which was brought about by the peak preovulatory concentrations of estradiol, occurred with a nadir of mean LH concentrations at 1000 to 3000 hours and an acrophase at 800 to 1000 hours. Because the midcycle LH surge in serum usually occurs during the early mornings, these investigators suggested that the critical concentrations of estradiol might be responsible for this phenomenon.

Jaffe and Keye[7] demonstrated previously that estradiol sensitizes the pituitary to GnRH. It is also likely that this phenomenon contributes to the initiation of the midcycle LH surge. Dumesic et al.[8] demonstrated that this estradiol sensitization of the pituitary gland to GnRH has its origins during intrauterine fetal life. Thus a large number of studies, including those noted above, indicate that the steroid milieu of the hypothalamic-pituitary system plays a critical role in the nature of GnRH and gonadotropin pulsatile discharge.

Opioids

The opioid peptides, particularly β-endorphin, inhibit pulsatile discharge of GnRH and LH. Opioid regulation of the GnRH pulse generator is probably central to the control of pulsatile discharge. Wardlaw et al.[9] demonstrated that long-term administration of estradiol plus progesterone markedly increased the concentration of β-endorphin in the hypothalamic-pituitary portal circulation of rhesus monkeys. The opiate antagonist, naloxone, causes an increase in pulsatile discharge of LH in women during the menstrual cycle that is greatest during the luteal phase of the cycle.[10] Morphine administration to castrate monkeys also inhibited pulsatile LH release, and this effect could be blocked by the administration of naloxone.[11] Thus opioids inhibit pulsatile discharge of GnRH, and the extent of this inhibition during the menstrual cycle is dependent on circulating levels of estradiol and progesterone.

Rasmussen et al.[1] showed that the pulsatile discharge of GnRH from the fetal and adult hypothalamus superfused in vitro is enhanced by the simultaneous administration of naloxone, which again suggests that regulation of pulsatile discharge of GnRH has its inception during pregnancy.

Corticotropin-releasing factor, which regulates the production of pituitary adrenocorticotropic hormone and β-endorphin, is increased during stress. The administration of corticotropin-releasing factor to castrate female rhesus monkeys inhibits pulsatile LH release.[12] This effect can be obviated by simultaneous long-term infusion of naloxone. These data suggest that corticotropin-releasing factor exerts its inhibitory action on pulsatile LH release through an opioidergic intermediary. A study by Barbarino et al.[13] during the midluteal phase of the menstrual cycle suggested that a similar mechanism of opioid modulation by corticotropin-releasing factor was operative in the inhibition of pulsatile LH discharge. These phenomena may be involved in the mechanism of stress-induced amenorrhea.

The administration of opioid receptor agonists, such as β-endorphin, enkephalin analogs, and morphine, increases serum prolactin in men and women. Concurrent with the increase in prolactin, declines in serum LH have been demonstrated in women during the early follicular phase in response to β-endorphin[14] and in postmenopausal women in response to an enkephalin analog[15] but not in postmenopausal women treated with β-endorphin.[16]

The lack of response of LH to β-endorphin in postmenopausal women may indicate that gonadotropin sensitivity to opioidergic modulation is dependent on the presence of adequate amounts of gonadal steroids. However, a decline in LH in response to an enkephalin analog in postmenopausal women not receiving steroid replacement has been observed.[15] Different biologic potencies, rates of metabolism, and different experimental conditions may have contributed to the observed differences.

The decrements in circulating LH seen in humans in response to opioid receptor agonists are qualitatively similar to those in rodents and nonhuman primates. The in vivo and in vitro evidence is that opioids can regulate gonadotropin secretion by inhibition of tuberoinfundibular GnRH secretion in humans by mechanisms similar to those in rodents and nonhuman primates. This evidence is more substantive than the evidence for a parallel mechanism of interaction between opioids and dopamine.

Gonadotropin response to opioid antagonists. The picture that has emerged from opiate antagonist studies is one in which endogenous opioid substances modulate pituitary LH secretion but only under physiologic conditions characterized by sufficient quantities of gonadal steroids (i.e., in the late follicular to midluteal

phases of the menstrual cycle).[17-19] The pattern of LH responses to naloxone is consistent with tonic endogenous opioidergic inhibition of GnRH secretion.

As in other species, opioid receptor blockade in humans results in increases in LH, although in healthy women this effect is limited to estrogenic or estrogenic-progestogenic environments.[18-22]

Response in different estrogen environments. In women, ovarian estrogen secretion is low during the early follicular phase. The administration of naloxone during this phase of the menstrual cycle does not bring about a change in gonadotropin levels,[23, 24] which indicates that estradiol levels are insufficient during the midfollicular phase to induce endogenous opioid tonic modulation of gonadotropins. However, in a study during the early follicular phase, significant increments in LH were observed in response to naltrexone.[25] These findings, in contrast to those in which naloxone was used, suggest a role for tonic endogenous opioid regulation of LH secretion during the early follicular phase. Different effects of naloxone and naltrexone may be the result of differences in potency, receptor binding, or purity as an antagonist.

The role of gonadal steroids in regulating endogenous opioid tone becomes apparent in the high estradiol environment of the late follicular phase. When naloxone was infused in the late follicular phase 3 days before the anticipated LH surge, half of the women had marked increases in LH pulse amplitude that resembled a spontaneous LH surge. The surge lasted 24 hours, and postsurge ovulation occurred. The other women did not respond. The group of nonresponders had estradiol levels significantly lower during naloxone infusion than on the day before the spontaneous surge in the antecedent control cycle. This suggests an obligatory role for estradiol in the regulation of endogenous opioid tone at this time of the cycle.[19] Because the characteristics of the LH surge in those women who responded to naloxone were similar to the characteristics of physiologic spontaneous LH surges, this study provides evidence that the initiation of the LH surge may be mediated by physiologic withdrawal of endogenous opioid tone in the presence of sufficient quantities of estradiol.

In the high estradiol-progesterone environment of the midluteal phase, naloxone administration was associated with increased LH pulse frequency.

Long-term opioid antagonism with naltrexone that was administered during the first week of the luteal phase produced subtle effects on LH. There was an increase in midluteal LH pulse frequency, but total LH secretion did not increase.[18] The less dramatic effects seen with long-term opioid antagonism compared with short-term naloxone infusion studies may reflect an ability of the central nervous system–GnRH-pituitary-ovarian axis to adapt to long-term opioid withdrawal.

The administration of conjugated estrogens to previously untreated postmenopausal women brought about a return of prolactin sensitivity to opioid receptor blockade. Estrogen therapy was then discontinued, and combined estrogen-progestin therapy was started 20 days later. After 20 days on the combination regimen, infusion of naloxone resulted in prolactin elevations to 245% above baseline levels. This was a significantly greater increment than that seen with estrogen replacement in the absence of a progestin.[21] Treatment of oophorectomized women with estrogen restores opioid activity.[26] Petraglia et al.[27] showed that both fertile and climacteric women had no LH response to naloxone by 1 week after ovariectomy. The combination of conjugated estrogens and progestins restored naloxone induction of the LH rise. These findings demonstrate that estrogen and progesterone together modulate endogenous opioid tone to a greater extent than either steroid alone.

Modulators of endogenous opioid tone

Dopamine. There is evidence that dopamine modulates endogenous opioid tone, raising the possibility of a feedback loop between hypothalamic opioids and catecholamines. Dopamine receptor antagonists increase levels of β-endorphin in the hypothalamus, pituitary, and peripheral serum of rodents.[28, 29] Naloxone studies also suggest that dopamine antagonism results in increases in endogenous opiate tone. In postmenopausal women experiencing vasomotor symptoms, treatment with the dopamine receptor antagonist, veralipride, brought about a decrease in basal LH levels[30] and a reduction in core temperature that is a characteristic effect of opioids.[31] Naloxone infusion after veralipride resulted in a rise in LH levels.[30] Naloxone infusion after veralipride resulted in a rise in levels in LH.[30] These findings suggested that long-term dopamine receptor blockade resulted in elevated endogenous opioid levels, which in turn decreased LH and increased response to naloxone.

The infusion of dopamine to women during the follicular phase of the menstrual cycle in concentrations that elevated peripheral dopamine concentrations only slightly above the physiologic range did not result in significant alterations in pulsatile LH discharge.[32]

With the use of higher pharmacologic doses, suppression of LH was observed that was particularly marked in the luteal phase of the cycle.[33] Recently Plosker et al.[34] administered the tyrosine hydroxylase inhibitor, α-methyl paratyrosine, to women during the follicular phase. This inhibited endogenous catecholamine synthesis and resulted in an increase in the

amplitude of LH pulses. Thus endogenous catecholamines, primarily dopamine, appear to inhibit pulsatile LH release. It is unknown whether this action occurs in the pituitary gland or the hypothalamic GnRH pulse generator directly, or if it is mediated by hypothalamic opioids. The study by Plosker et al. suggests a hypothalamic locus of action.

Steroids The observations from experiments with naloxone and naltrexone in reproductive-age women during different intervals in the menstrual cycle and in menopausal women given pharmacologic steroid replacement imply a significant role for gonadal steroids in the modulation of endogenous opioid tone. Research on rodents and nonhuman primates has further defined this role. Steroid hormone receptors are widespread in the central nervous system, including regions of the hypothalamus, where opioid peptides, GnRH, and catecholaminergic neurons are localized.[35, 36] The neuroanatomic relationships for estrogens, progesterone, and androgens to modulate opioid-catecholamine or GnRH regulation of pituitary hormone secretion exist. Factors, such as the dose of steroid administered, the multiple sites of action of steroids,[37] the cyclic fluctuations in steroid secretion, and the interactions between steroids, often make it difficult to interpret steroidal effect.

Ovarian sex steroids appear essential for the release of β-endorphin into pituitary stalk blood, with the greater amounts of β-endorphin released in the presence of both estrogen and progesterone.[9] Also, opioid peptides probably have a role in mediating gonadal steroid feedback at the hypothalamic level. Some investigators have postulated that an alteration in opioid peptides, which are modulated by sex steroids, may be responsible for the cyclic nature of the menstrual cycle.[38-40]

Concentrations of β-endorphin in the peripheral circulation are extremely low, and hypothalamic β-endorphin activity is better reflected by direct measurements of the hormone in pituitary stalk blood. Pituitary stalk β-endorphin concentration in monkeys is high in the mid- to late follicular phase, higher in the luteal phase, and undetectable after ovariectomy or during menstruation.[41] Long-term estrogen administration to ovariectomized monkeys restored β-endorphin secretion, and the addition of progesterone further elevated β-endorphin levels.[9] In nonhuman primates ovarian sex steroids are necessary for the release of hypothalamic β-endorphin into the pituitary portal circulation.

The presence of exogenous gonadal steroids is not sufficient to activate the opioidergic control of LH secretion. A functional hypothalamic GnRH-pituitary-gonadal system from spontaneous pubertal development also seems to be necessary. The administration of an estrogen-progestin combination to patients with Turner's syndrome did not affect their response to naloxone.[42]

Gonadotropin responses to naloxone infusions in low estrogen states such as hypothalamic amenorrhea, and the absence of response in polycystic ovarian syndrome, a condition that is not characterized by low levels of estrogen, imply that factors in addition to gonadal steroids influence endogenous opioid tone.

Thus there is a complex, interdependent, multifaceted interaction of steroids, opioids, catecholamines, and other neurotransmitters in the regulation of GnRH and gonadotropin pulsatile discharge in women.

REFERENCES

1. Rasmussen D, Liu J, Wolf P, et al. Endogenous opioid regulation of gonadotropin-releasing hormone release from human fetal hypothalamus in vitro. J Clin Endocrinol Metab 1983;57:881.
2. Martin MC, Hofmann J, Voytek C, Monroe SE, Jaffe RB. Pulsatile luteinizing hormone secretion by the primate fetus in utero. [Abstract]. 37th Annual Meeting of the Society for Gynecologic Investigation, 1990, No. 52:122.
3. Midgley AR Jr, Jaffe RB. Regulation of human gonadotropins. X. Episodic fluctuation of LH during the menstrual cycle. J Clin Endocrinol Metab 1971;33:962.
4. Yen SSC, Tsai CC, Naftolin F, Vandenberg G, Ajabor L. Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. J Clin Endocrinol Metab 1972;34:671.
5. Soules MR, Steiner RA, Cohen NL, Bremner WJ, Clifton DK. Nocturnal slowing of pulsatile luteinizing hormone secretion in women during the follicular phase of the menstrual cycle. J Clin Endocrinol Metab 1985;61:43.
6. Marshall L, Martin M, Jaffe R. Effect of preovulatory estradiol concentrations on luteinizing hormone diurnal secretory patterns: a hypothesis for the timing of the luteinizing hormone surge. Am J Obstet Gynecol 1988; 159:1123.
7. Jaffe RB, Keye WR Jr. Estradiol augmentation of pituitary responsiveness to gonadotropin-releasing hormone in women. J Clin Endocrinol Metab 1974;39:850.
8. Dumesic DA, Goldsmith PC, Jaffe RB. Estradiol sensitization of cultured human fetal pituitary cells to gonadotropin releasing hormone. J Clin Endocrinol Metab 1987; 65:1147.
9. Wardlaw S, Wehrenberg W, Ferin M, et al. Effect of sex steroids on beta-endorphin in hypophyseal portal blood. J Clin Endocrinol Metab 1982;55:877.
10. Yen SSC, Quigley M, Reid R, et al. Neuroendocrinology of opioid peptides and their role in the control of gonadotropin and prolactin regulation. Am J Obstet Gynecol 1985;152:485.
11. Gold M, Redmond D, Donabedian R. The effects of opiate agonist and antagonist on serum prolactin in primates: possible role for endorphins in prolactin regulation. Endocrinology 1979;105:284.
12. Olster DH, Ferin M. Corticotropin-releasing hormone inhibits gonadotropin secretion in the ovariectomized rhesus monkey. J Clin Endocrinol Metab 1987;65:262.
13. Barbarino A, De Marinis L, Tofani S, et al. Corticotropin-releasing hormone inhibition of gonadotropin release and the effect of opioid blockade. J Clin Endocrinol Metab 1989;68:523.
14. Reid R, Hoff J, Yen SSC. Effects of exogenous beta-endorphin on pituitary hormone secretion and its disappearance in normal human subjects. J Clin Endocrinol Metab 1981;52:1179.

15. Grossman A, Moult P, Gaillard R, et al. The opioid control of LH and FSH release: effects of met-enkephalin analogue and naloxone. Clin Endocrinol (Oxford) 1981;14: 381.
16. Reid R, Quigley M, Yen SSC. The disappearance of opioidergic regulation of gonadotropin secretion in postmenopausal women. J Clin Endocrinol Metab 1983;57: 1107.
17. Cetel N, Quigley M, Yen SSC. Naloxone-induced prolactin secretion in women; evidence against a direct prolactin stimulatory effect of endogenous opioids. J Clin Endocrinol Metab 1985;60:191.
18. Gindoff P, Jewelwicz R, Hembree W, et al. Sustained effects of opioid antagonism during the normal luteal phase. J Clin Endocrinol Metab 1988;66:1000.
19. Rossmanith W, Mortola J, Yen SSC. Role of endogenous opioid peptides in the initiation of the mid-cycle luteinizing hormone surge in normal cycling women. J Clin Endocrinol Metab 1988;67:695.
20. Casper R, Alapin-Rubillovitz S. Progestins increase endogenous opioid activity in post-menopausal women. J Clin Endocrinol Metab 1985;60:34.
21. Melis G, Gambacciani M, Paoletti A, et al. Sex steroids modulate response to naloxone in post-menopausal women. Neuroendocrinology 1985;41:138.
22. Casper R, Bhanot R, Wilkinson M. Prolonged elevation of hypothalamic opioid peptide activity in women taking oral contraceptives. J Clin Endocrinol Metab 1984;58:582.
23. Quigley M, Sheehan K, Casper R, et al. Evidence for an increased opioid inhibition of luteinizing hormone secretion in hyperprolactinemic patients with pituitary microadenoma. J Clin Endocrinol Metab 1980;50:427.
24. Quigley M, Sheehan K, Casper R, et al. Evidence for increased dopaminergic and opioid activity in patients with hypothalamic hypogonadotrophic amenorrhea. J Clin Endocrinol Metab 1980;50:949.
25. Teoh S, Mendelson J, Mello N, et al. Alcohol effects on naltrexone-induced stimulation of pituitary, adrenal, and gonadal hormones during the early follicular phase of the menstrual cycle. J Clin Endocrinol Metab 1988;66:1181.
26. Shoupe D, Montz F, Lobo R. The effects of estrogen and progestin on endogenous opioid activity in oophorectomized women. J Clin Endocrinol Metab 1985;60:178.
27. Petraglia F, Comitini G, D'Ambrogio G, et al. Short-term effects of ovariectomy: the opioid control of LH secretion in fertile, climacteric, and postmenopausal women. J Endocrinol Invest 1985;8:325.
28. Hollt V, Bergmann M. Effects of acute and chronic haloperidol treatment on the concentrations of immunoreactive beta-endorphin in plasma, pituitary, and brain of rats. Neuropharmacology 1982;21:147.
29. Nohtomi A, Itoh M, Yufu N. Effects of sulpiride treatment on the levels of immunoreactive beta-endorphin in rat hypothalamic nuclei. Brain Res 1984;300:152.
30. Melis G, Cagnacci A, Gambacciani M, et al. Restoration of luteinizing hormone response to naloxone in postmenopausal women by chronic administration of the antidopaminergic drug veralipride. J Clin Endocrinol Metab 1988;66:694.
31. Cagnacci A, Melis G, Paoletti A, et al. Interaction between veralipride and the endogenous opioid system in the regulation of body temperature in postmenopausal women. Life Sci 1988;42:547.
32. Martin M, Monroe S, Weiner RI, Jaffe RB. Low-dose dopamine infusions do not ablate luteinizing hormone pulses in women. AM J OBSTET GYNECOL 1988;159:898.
33. Yen SSC. The human menstrual cycle. In: Yen SCC, Jaffe RB, eds. Reproductive endocrinology—physiology, pathophysiology and clinical management. Philadelphia: WB Saunders Co, 1986:222.
34. Plosker S, Rabinovici J, Jaffe R. Endogenous catecholamines inhibit early follicular phase LH secretion. [Abstract]. 37th Annual Meeting of the Society for Gynecologic Investigation, 1990, No. 385:289.
35. MacLuskey N, Lieberburg I, Krey L, et al. Progestin receptors in the brain and pituitary of the bonnet monkey *(Macaca radiata)*: differences between the monkey and the rat in the distribution of progestin receptors. Endocrinology 1980;106:185.
36. Pfaff D, Gerlach J, McEwen B, Ferin M, Carmel P, Zimmerman E. Autoradiographic localization of hormone-concentrating cells in the brain of the female rhesus monkey. J Comp Neurol 1976;170:279.
37. Chappel S, Resko J, Norman R, et al. Studies in rhesus monkeys on the site where estrogen inhibits gonadotropins: delivery of 17 beta-estradiol to the hypothalamus and pituitary gland. J Clin Endocrinol Metab 1981;52:1.
38. Blankstein J, Reyes F, Winter J, et al. Endorphins and the regulation of the human menstrual cycle. Clin Endocrinol (Oxford) 1981;14:287.
39. Ferin M, Van de Wiele R. Endogenous opioid peptides and control of the menstrual cycle. Eur J Obstet Gynecol Reprod Biol 1984;18:365.
40. Ferin M, Van Vugt D, Wardlaw S. The hypothalamic control of the menstrual cycle and the role of endogenous opioids. Rec Prog Horm Res 1984;40:441.
41. Wehrenberg W, Wardlaw S, Frantz A, et al. Beta-endorphin in hypophyseal portal blood: variations throughout the menstrual cycle. Endocrinology 1982; 111:879.
42. Petraglia F, Larizza D, Maghnie M, et al. Impairment of opioidergic control of luteinizing hormone secretion in Turner's syndrome: lack of effect of gonadal steroid therapy. J Clin Endocrinol Metab 1988;66:1024.

Hypothalamic amenorrhea: Clinical perspectives, pathophysiology, and management

James H. Liu, MD
Cincinnati, Ohio

The development of functional hypothalamic amenorrhea reflects an individual's response to environmental stressors and life-style variables. In this disorder there are no detectable anatomic abnormalities with respect to the hypothalamic-pituitary-ovarian-endometrial axis. Current evidence suggests that the common underlying defect is a decrease in the activity of the hypothalamic gonadotropin-releasing hormone pulse generator. Of the neuroendocrine factors that appear to regulate gonadotropin-releasing hormone activity, the opiate and dopamine neuronal systems have been implicated as factors that are responsible in part for the decreased secretion of gonadotropin-releasing hormone. Because of the functional nature of this disorder, reactivation of the hypothalamic-pituitary unit would be expected to take place in most women after accommodation to environmental stressors or modification in life-style. For women with persistent anovulation, treatment with estrogen-replacement therapy should be offered. In those desiring fertility, ovulation induction with pulsatile gonadotropin-releasing hormone would be the most effective modality. (AM J OBSTET GYNECOL 1990;163:1732-6.)

Key words: Hypothalamic amenorrhea, gonadotropin-releasing hormone, environmental stressors, opiate, dopamine

Amenorrhea associated with hypoestrogenism, normal or low levels of follicle-stimulating hormone (FSH), and psychogenic factors was originally described by Klinefelter et al.[1] in 1973. They were the first to coin the term "hypothalamic hypoestrinism" and postulated that this disorder was caused by the "failure of the hypothalamic-pituitary nervous pathways to release luteinizing hormone from the anterior pituitary." In recent years many studies have shown that individuals with hypothalamic amenorrhea have intact pituitary-ovarian-endometrial function[2-4] and the common underlying defect in this disorder is a deficiency in pulsatile secretion of gonadotropin-releasing hormone (GnRH).[5-7] Unfortunately, the neuroendocrine basis that can account for the decrease in GnRH-luteinizing hormone (LH) secretion remains unclear. An increasing number of studies have provided evidence that increased endogenous opiate activity plays a major role in suppressing pulsatile GnRH secretion.[6, 8] In addition, other studies have suggested that there is a functional link between environmentally and endogenously generated stress and onset of hypothalamic amenorrhea.[1, 3, 9] With our alterations in life-style and the growing emphasis on exercise, nutrition, and slenderness, the incidence of hypothalamic amenorrhea can be expected to increase.

From the Department of Obstetrics and Gynecology, University of Cincinnati.
Reprint requests: James H. Liu, MD, Department of Obstetrics and Gynecology, University of Cincinnati, 231 Bethesda Ave., ML 526, Cincinnati, OH 45267-0526.
6/0/24547

Clinical and biochemical features of hypothalamic amenorrhea

On a clinical basis it is difficult to provide a typical profile of the individual with hypothalamic amenorrhea because functional hypothalamic amenorrhea is considered a diagnosis of exclusion. There are several other related disorders that can be considered to be caused by dysfunction of the central nervous-hypothalamic-pituitary system (Table I).

One such disorder, isolated gonadotropin deficiency, shares many of the biochemical features with functional hypothalamic amenorrhea. Isolated gonadotropin deficiency is characterized by a decrease in the secretion of endogenous GnRH leading to hypogonadotropic hypogonadism, eunuchoid features, incomplete development of secondary sexual characteristics, primary amenorrhea, and, in some cases, anosmia. Because this defect is probably the result of a failure of GnRH neurons to develop completely or migrate from the olfactory bulb to the hypothalamus during embryogenesis, hypoplasia of the olfactory bulbs can be identified in some patients.[10]

In contrast, patients with a diagnosis of functional hypothalamic amenorrhea can be defined as those who have an absence of menstrual cycles for more than 6 months in which no anatomic or organic abnormalities can be identified. Most women with this disorder have a history of normal onset of menarche with regular menstrual cycles between 26 and 35 days. These patients tend to be highly motivated, intelligent, involved in high-stress occupations, and usually thin or of normal body weight. In some patients a variety of emo-

Table I. Classification of anovulation caused by the central nervous-hypothalamic-pituitary system

Functional hypothalamic anovulation
Psychogenic or stress factors
Nutritional factors
Exercise-related factors
Physiologic anovulation
Prepubertal period
Postpartum period
Pharmacologic-induced anovulation
Dopaminergic antagonist
Opiate agonist
Psychiatric-associated disorders
Anorexia nervosa
Pseudocyesis
Organic defects of the hypothalamic-pituitary unit
Isolated gonadotropin deficiency
Pituitary tumors
Sheehan's syndrome
Empty-sella syndrome
Head trauma
Inappropriate prolactin secretion

tional or stressful events (e.g., divorce) preceding the onset of amenorrhea can be identified during the initial interview. Other environmental and interpersonal factors that can be uncovered include academic pressure, social maladjustment, and psychosexual difficulties. Life-style factors that should be scrutinized include a history of weight fluctuations, dietary habits, type and intensity of exercise, and use of sedatives and hypnotics.

A thorough physical examination should reveal normal secondary sexual development and fail to demonstrate abnormalities such as galactorrhea, thyroid enlargement, or signs of androgen excess. Despite findings of hypoestrogenic changes in the genital tract, these patients do not usually complain of hot flashes.

Laboratory values including LH, FSH, prolactin, and thyroid-stimulating hormone levels are in the low or normal range (Table II).[7] Estradiol levels are typically low or low normal. In general the progestin challenge test should confirm that there is a scant or absent estrogenic effect on the endometrium. To exclude an occult pituitary lesion, computerized tomographic scanning of the sella turcica should be performed.

Pulsatile GnRH-LH secretion in hypothalamic amenorrhea

Findings from several laboratories have established that there is a slowing in frequency of hypothalamic GnRH-LH secretion that is the basic underlying cause of persistent anovulation in women with hypothalamic amenorrhea.[5-7, 11] In these studies frequent peripheral blood samples were used to quantitate pulsatile LH secretion and provide an indirect assessment of GnRH secretion. These studies make the assumption that only

Table II. Basal serum hormonal profiles in women with functional hypothalamic amenorrhea

	Hypothalamic amenorrhea	*Early follicular phase*
LH (units/L)	8.5 ± 1.1*	11.6 ± 1.2
FSH (units/L)	9.3 ± 0.5*	12.1 ± 1.0
Prolactin (μg/L)	12.2 ± 0.8†	17.1 ± 1.4
Thyroid-stimulating hormone (mU/L)	1.05 ± 0.33	1.33 ± 0.26
Growth hormone (μg/L)	6.7 ± 1.3	4.2 ± 0.7
Estradiol (pmol/L)	142 ± 15	156 ± 10

From Berga SL, Mortola JF, Girton L, et al. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 1989;68:301.

Date are mean ± SE.

*$p < 0.05$.

†$p < 0.01$.

GnRH is capable of stimulating LH secretion and there is a close correlation between GnRH and LH secretion, as demonstrated previously in several animal models.[12, 13]

When exogenous GnRH is administered to women with hypothalamic amenorrhea, LH and FSH responses are either normal or exaggerated compared with women with eumenorrhea.[2, 14] These findings suggest that pituitary sensitivity and capacity are not impaired and the primary defect is at a central level. Examination of individual patterns of LH secretion demonstrates considerable variability in the pulsatile pattern (Fig. 1). Compared with the follicular phase of the cycle, these LH secretory patterns are characterized by alterations in LH frequency, amplitude, and, in some cases, a regression to a pubertal pattern.[5, 6] These LH patterns suggest that the activity of the GnRH pulse generator is not uniformly suppressed to the same degree in each individual. During recovery from hypothalamic amenorrhea, a reversal of the GnRH-LH secretory pattern, characterized by a sleep-associated increase in LH amplitude, is often evident.

Opioidergic and dopaminergic modulation of GnRH secretion

A growing body of evidence indicates that endogenous opiates inhibit pulsatile GnRH and LH secretion.[15, 16] In a subgroup of women with hypothalamic amenorrhea, blockade of endogenous opiate receptors by naloxone can increase LH pulse frequency and amplitude.[6, 8] In addition, long-term treatment with the opiate receptor antagonist naltrexone leads to a spontaneous return of gonadotropin secretion and ovulatory function.[17] Collectively these studies suggest that an increase in endogenous opiate activity plays a significant role in reducing pulsatile GnRH secretion in this disorder.

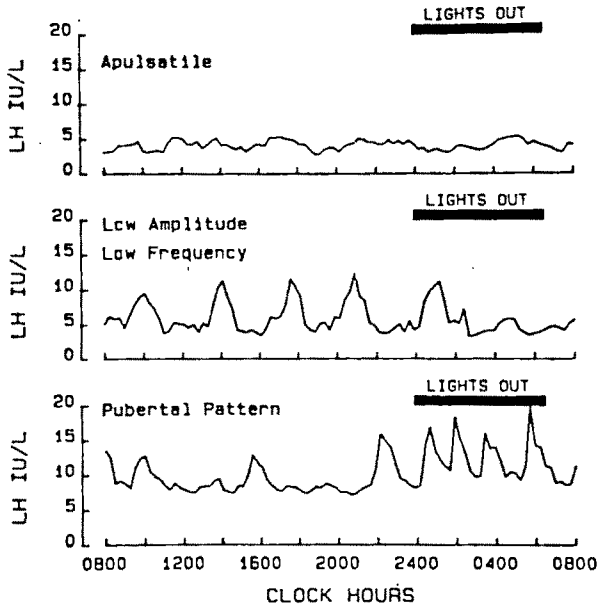


Fig. 1. Examples of the pulsatile pattern of LH in women with hypothalamic amenorrhea. Alterations in LH amplitude, frequency, and augmentation of LH secretion during the nocturnal hours are evident.

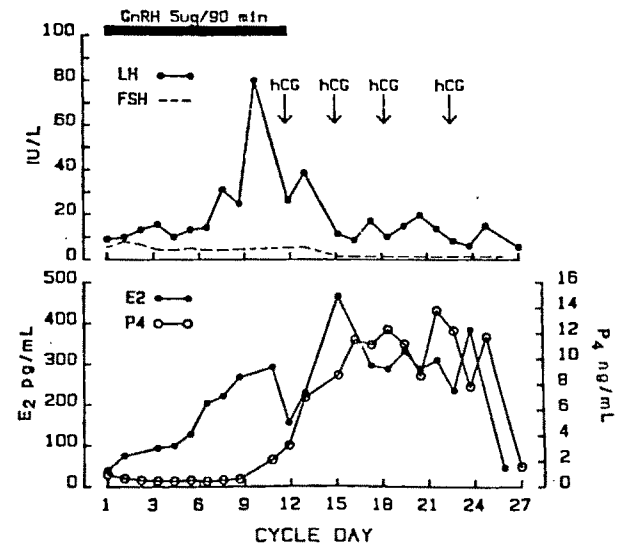


Fig. 2. LH, FSH, estradiol (E_2), and progesterone (P_4) levels during ovulation induction with intravenous pulsatile GnRH. For luteal-phase support, human chorionic gonadotropin *(hCG)*, 1500 units intramuscularly, is administered every 3 days for four doses.

Table III. Associated neuroendocrine abnormalities in hypothalamic amenorrhea

Increased amplitude and duration of nocturnal melatonin secretion[18]
Decreased 24 hr prolactin secretion with exaggerated nocturnal elevation[7]
Increased nocturnal secretion of growth hormone[7]
Blunted elevation in prolactin, adrenocorticotropic hormone, and cortisol levels with meals[9, 14]

Using a similar pharmacologic approach, Quigley et al.[8] have also demonstrated that blockade of the dopamine receptor with metaclopramide can induce an increase in LH secretion. These findings indirectly suggest that, in some patients with hypothalamic amenorrhea, there is enhanced dopaminergic activity. Because the dopaminergic and opioidergic neuronal systems are also involved in the regulation of other hypothalamic hormones, it is not surprising that there are altered secretion patterns of other hormones such as growth hormone, prolactin, adrenocorticotropic hormone, cortisol, and melatonin in women with hypothalamic amenorrhea (Table III).[7, 18]

Stress and reproductive function

The disruption of reproductive function during chronic exposure to stress has been reported in both animal species and humans.[1, 3, 19] According to Selye's original observations,[20] stress is met by increased hormonal secretions of the hypophyseal-adrenocortical axis. These effects on the reproductive axis by the hypothalamic-pituitary-adrenal axis appear to be mediated at several levels. At the hypothalamic level administration of corticotropin-releasing hormone in rats and monkeys acutely inhibits GnRH-LH release in vivo[19, 21, 22] and in vitro.[23] This inhibitory effect can be prevented by the coadministration of a corticotropin-releasing hormone receptor antagonist or reversed by naloxone, which suggests that the action of corticotropin-releasing hormone is mediated through the opioidergic system.[19, 22]

At the pituitary level the administration of adrenocorticotropic hormone can delay pubertal maturation and disrupt the reproductive cycle in adrenalectomized rats.[23] In addition, chronic hypercortisolism leads to a decrease in pituitary sensitivity to GnRH.[24]

In humans there is evidence that links stress, chronic activation of the hypothalamic-pituitary-adrenal axis, and reproductive dysfunction in athletes and women with hypothalamic amenorrhea.[9, 14] In these women there is a significant elevation in daytime cortisol levels, a delay or absent response in adrenocorticotropic hormone and cortisol secretion during the noon meal, and a blunted response to corticotropin-releasing hormone.[9, 14] These clinical studies suggest that there is a functional association between chronic stress and the onset of ovulatory dysfunction and amenorrhea.

Management of hypothalamic amenorrhea

Because of the functional nature of hypothalamic amenorrhea, a major emphasis should be placed on a

carefully conducted initial interview that focuses on life-style variables and interpersonal relationships. Significant organic disease must be excluded. In many instances spontaneous recovery of menstrual function will return after reassurance, psychologic guidance, and modification of life-style.[3] For this reason, expectant management remains a viable option. Periodic reassessment of reproductive status in these individuals (i.e., every 4 to 6 months) would be prudent.

For those women with persistent anovulation, a major concern is the long-term effect of hypoestrogenism on bone density. Unfortunately, there is very little information with regard to this aspect of hypothalamic amenorrhea from an epidemiologic perspective.[25] Based on our current understanding of bone mineral metabolism, chronic estrogen deprivation can lead to significant osteopenia that will predispose these patients to the increased risk of bone fractures. Because these patients are often reluctant to take medications, serial bone density studies may be necessary to convince them of the necessity to begin estrogen-replacement therapy.

For women who are interested in fertility, a trial of clomiphene citrate is indicated. Because clomiphene citrate displays weak estrogenic properties in a hypoestrogenic environment,[26] lower doses of clomiphene (25 mg/day for 5 days) should be used initially. In some cases higher doses of clomiphene (>100 mg/day) may exert a paradoxic effect and suppress the hypothalamic-pituitary axis.

For those women who fail to respond to clomiphene, the most logical approach would be ovulation induction with pulsatile GnRH. Because the primary underlying defect in hypothalamic amenorrhea is decreased endogenous GnRH secretion, this approach can be viewed as a form of "physiologic" replacement therapy. For women with hypothalamic amenorrhea a starting intravenous dose of GnRH of 1 to 5 μg/90 min has been shown to be effective.[27,28] Monitoring of serum estradiol levels or follicular development can be minimized because ovarian follicular response and gonadotropin output mimic the natural menstrual cycle (Fig. 2). Gonadotropin support of the corpus luteum can be maintained with either continuation of pulsatile GnRH or human chorionic gonadotropin, 1500 units intramuscularly every 3 days for four doses. With the intravenous mode of GnRH therapy, ovulation rates of approximately 90%, conception rates of 25% to 30% per ovulatory cycle, and hyperstimulation rates of less than 1% have been reported in patients with hypothalamic amenorrhea.[29]

In summary, the development of functional hypothalamic amenorrhea is linked to life-style variables and environmental stressors. From a teleologic perspective, the reproductive cycle shifts into a temporary "resting phase" characterized by decreased secretion of endogenous GnRH under these unfavorable conditions. Neuroendocrine factors, which appear to modulate reduced GnRH activity in this disorder, include the opiate and dopamine neuronal systems. In most women spontaneous reactivation of the hypothalamic-pituitary unit will take place after accommodation to stress factors or modification in life-style.

REFERENCES

1. Klinefelter HF Jr, Albright F, Griswold GC. Experience with a quantitative test for normal or decreased amounts of follicle-stimulating hormone in urine in endocrinological diagnosis. J Clin Endocrinol Metab 1973;3:529.
2. Yen SSC, Rebar R, VandenBerg G, Judd H. Hypothalamic amenorrhea and hypogonadotropism: responses to synthetic LRF. J Clin Endocrinol Metab 1973;36:811.
3. Lachelin GCL, Yen SSC. Hypothalamic chronic anovulation. Am J Obstet Gynecol 1978;130:825.
4. Rebar RW, Harman SM, Vaitukaitis JL. Differential responsiveness for LRF after estrogen therapy in women with hypothalamic amenorrhea. J Clin Endocrinol Metab 1978;46:48.
5. Reame NE, Sauder SE, Case GD, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion in women with hypothalamic amenorrhea: evidence that reduced frequency of gonadotropin secretion is the mechanism of persistent anovulation. J Clin Endocrinol Metab 1985;61:851.
6. Khoury SA, Reame NE, Kelch RP, Marshall JC. Diurnal patterns of pulsatile luteinizing hormone secretion in hypothalamic amenorrhea: reproducibility and response to opiate-blockade and an alpha$_2$-adrenergic agonist. J Clin Endocrinol Metab 1987;64:755.
7. Berga SL, Mortola JF, Girton L, et al. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 1989;68:301.
8. Quigley ME, Sheehan KL, Casper RF, Yen SSC. Evidence for increased dopaminergic and opioid activity in patients with hypothalamic hypogonadotropic amenorrhea. J Clin Endocrinol Metab 1980;50:949.
9. Suh BY, Liu JH, Berga SL, Quigley ME, Laughlin GA, Yen SSC. Hypercortisolism in patients with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 1988; 66:733.
10. Schwanzel-Fukuda M, Pfaff DW. Origin of luteinizing hormone–releasing hormone neurons. Nature 1989;338: 161.
11. Crowley WF Jr, Filicori M, Spratt D, Santoro N. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. Recent Prog Horm Res 1985;41:473.
12. Clarke IJ, Cummins JT. The temporal relationship between gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology 1982;111:1737.
13. Levine JG, Paw KF, Ramirez VD, Jackson GL. Simultaneous measurement of luteinizing hormone–releasing hormone release in unanesthetized, ovariectomized sheep. Endocrinology 1982;111:1449.
14. Loucks AB, Mortola JF, Girton L, Yen SSC. Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axis in athletic women. J Clin Endocrinol Metab 1989;68:402.
15. Blank MS, Panerai AE, Friesen HG. Opioid peptides modulate luteinizing hormone secretion during sexual maturation. Science 1979;203:1129.
16. Ropert JF, Quigley ME, Yen SSC. Endogenous opiates modulate pulsatile luteinizing hormone release in humans. J Clin Endocrinol Metab 1981;52:583.
17. Wildt L, Leyendecker G. Induction of ovulation by the

chronic administration of naltrexone in hypothalamic amenorrhea. J Clin Endocrinol Metab 1987;64:1334.
18. Berga SL, Mortola JF, Yen SSC. Amplification of nocturnal melatonin secretion in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 1988; 66:242.
19. Rivier C, Rivier J, Vale W. Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. Science 1986;231:607.
20. Selye H. The stress syndrome. Nature 1936;138:32.
21. Petraglia F, Sutton S, Vale W, Plotsky P. Corticotropin-releasing factor decreases plasma LH levels in female rats by inhibiting GnRH release into the hypophyseal portal circulation. Endocrinology 1987;120:1083.
22. Xiao E, Luckhaus J, Niemann W, Ferin M. Acute inhibition of gonadotropin secretion by corticotropin-releasing hormone in the primate: are the adrenal glands involved? Endocrinology 1989;124:1632.
23. Gambacciani M, Yen SSC, Rasmussen DD. GnRH release from the mediobasal hypothalamus: in vitro inhibition by corticotropin-releasing factor. Neuroendocrinology 1986; 43:533.
24. Mann DR, Jackson GG, Blank MS. Influence of adrenocorticotropin and adrenalectomy on gonadotropin secretion in immature rats. Neuroendocrinology 1982;34:20.
25. Drinkwater BL, Nilson K, Chestnut CH III, Bremner WJ, Shainholtz S, Southworth MB. Bone mineral content of amenorrheic and eumenorrheic athletes. N Engl J Med 1984;311:277.
26. Hsueh AJW, Erikson GF, Yen SSC. Sensitization of pituitary cells to luteinizing hormone–releasing hormone by clomiphene citrate in vitro. Nature 1978;273:57.
27. Liu JH, Yen SSC. The use of gonadotropin-releasing hormone for the induction of ovulation. Clin Obstet Gynecol 1984;27:975.
28. Reid RL, Fretts R, Van Vugt DA. The theory and practice of ovulation induction with gonadotropin-releasing hormone. AM J OBSTET GYNECOL 1988;158:176.
29. Blunt SM, Butt WR. Pulsatile GnRH therapy for the induction of ovulation in hypogonadotropic hypogonadism. Acta Endocrinol Suppl (Copenh) 1988;288:58.

Discussion

Audience. How do you normally maintain the luteal phase?

Dr. Liu. You tend to have fewer problems with the intravenous sites if you limit the infusion duration of intravenous GnRH to 14 to 16 days. Also, if you plan to use intravenous GnRH in consecutive cycles, you do not want to exhaust potential intravenous sites. For these reasons, we tend to use hCG to maintain the luteal phase.

Audience. How did you determine the correct dose and schedule for hCG in the luteal phase?

Dr. Liu. First, we calculated, on the basis of half-life of exogenous hCG, how long we needed to give hCG and at what intervals. Every 3 days was an approximation. We determined that 1500 units was the minimum dose that could maintain progesterone production in a simulated luteal phase.

Polycystic ovary syndrome: Abnormalities and management with pulsatile gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs

Marco Filicori, MD, Carlo Flamigni, MD, Elisabetta Campaniello, MD, Maria Cristina Meriggiola, MD, Laura Michelacci, MD,[a] Alessandro Valdiserri, MD, and Paola Ferrari, BSc

Bologna, Italy

Ovulation induction with pulsatile gonadotropin-releasing hormone achieves high ovulatory and pregnancy rates in hypogonadotropic hypogonadism while limiting the occurrence of ovarian hyperstimulation and multiple pregnancy. However, this form of therapy is apparently less effective in polycystic ovary syndrome. The administration of a gonadotropin-releasing hormone analog for 4 to 8 weeks before the initiation of pulsatile gonadotropin-releasing hormone ovulation induction can temporarily correct endocrine abnormalities of polycystic ovary syndrome, such as excessive luteinizing hormone and androgen secretion, and improve ovulatory and pregnancy rates in these patients. For optimal results, this pretreatment should probably be repeated before each pulsatile gonadotropin-releasing hormone ovulation induction cycle. Obesity is associated with a lower success rate, and spontaneous abortion remains a prominent complication in polycystic ovary syndrome even after gonadotropin-releasing hormone analog suppression. With this regimen the risks of ovarian hyperstimulation and multiple pregnancy are virtually abolished. Thus, pulsatile gonadotropin-releasing hormone appears to be highly effective and safe for ovulation induction in patients with polycystic ovary syndrome also, provided that this treatment is preceded by pituitary-ovarian suppression with a gonadotropin-releasing hormone analog. (AM J OBSTET GYNECOL 1990;163:1737-42.)

Key words: Pulsatile gonadotropin-releasing hormone, polycystic ovary syndrome, GnRH analog, ovulation induction

Polycystic ovary syndrome is a disorder frequently encountered among patients seen in gynecologic and reproductive endocrine clinics. Common complaints of polycystic ovary syndrome include hirsutism, obesity, menstrual disorders (mainly oligomenorrhea), and infertility. Anovulation is common and ovulation induction is often resorted to for treatment of infertility in this disorder.

Several hormones are secreted abnormally in polycystic ovary syndrome (Fig. 1). Excessive androgen levels are common and cause acne and hirsutism; elevated intraovarian androgens also affect folliculogenesis and may participate in the pathogenesis of anovulation.[1] Although large amounts of excessive androgens and androgen substrate appear to be produced by the ovary, the adrenal gland may contribute substantially to hyperandrogenism in polycystic ovary syndrome.[2] A high pituitary output of luteinizing hormone (LH) is a frequent finding in polycystic ovary syndrome. The episodic secretion of LH is deranged; LH peaks are of greater amplitude than in normal women in the follicular phase of the menstrual cycle.[3-6] Excessive frequency of gonadotropin-releasing hormone (GnRH)–induced LH pulses may also be present,[3, 4, 7] although this feature is still the subject of some controversy.[5, 6] Pituitary sensitivity is also increased so that large amounts of gonadotropins are secreted when the pituitary gland is challenged with exogenous GnRH[8]; this derangement is probably responsible for the elevation of LH pulse amplitude present in polycystic ovary syndrome.

Excessive androgen levels indirectly affect gonadotropin secretion through various mechanisms. Androstenedione is peripherally converted to estrone; androgens also lower sex hormone–binding globulin levels so that increased amounts of unbound androgens and unbound 17β-estradiol are present in polycystic ovary syndrome.[9] These high estrone and free 17β-estradiol levels may act at the pituitary level to increase LH and follicle-stimulating hormone (FSH) reserve. This vicious cycle of high LH output, excessive androgen secretion, and elevated weak estrogen levels participates critically in the maintenance of deranged hor-

From the Center for Chronobiology of Reproduction, Reproductive Medicine Unit, and Prenatal Diagnosis Unit,[a] Department of Obstetrics and Gynecology, University of Bologna.
Reprint requests: Marco Filicori, MD, Fisiopatologia Riproduzione, Clinica Ostetrica e Ginecologica, Via Massarenti 13, 40138 Bologna, Italy.
6/0/24545

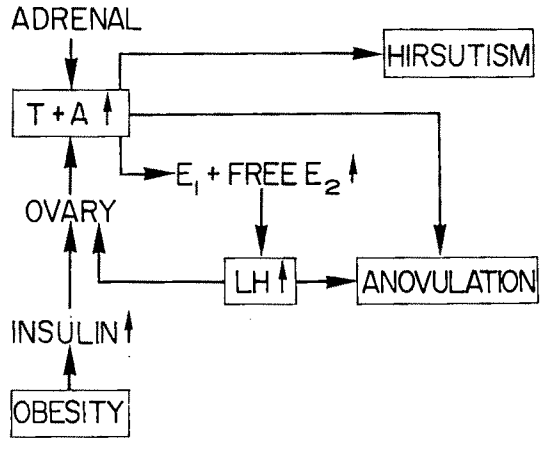


Fig. 1. Schematic representation of the clinical and endocrine abnormalities encountered in polycystic ovary syndrome. *T*, Testosterone; *A*, androstenedione; E_1, estrone; E_2, estradiol.

monal dynamics and the pathogenesis of anovulation in polycystic ovary syndrome.

Obesity may further complicate the endocrine picture in polycystic ovary syndrome. Obese patients with polycystic ovary syndrome secrete excessive amounts of insulin that may interact with insulin-like growth factor receptors in the ovary.[10] The synergistic action of LH and insulin may worsen ovarian androgen secretion and affect folliculogenesis. Weight loss is associated with a resumption of more regular menstrual function in women with polycystic ovary syndrome.[11]

Treatment of polycystic ovary syndrome

The specific cause of polycystic ovary syndrome is still obscure; furthermore, rather than being a single entity, polycystic ovary syndrome can be the end point of several endocrine disorders. Thus, physicians are often forced to treat symptoms instead of being able to solve the basic pathogenetic mechanism involved in this disorder. Drugs commonly used in the treatment of hirsutism include estrogen/progestogen combinations,[12] cyproterone acetate,[13] and spironolactone.[14] With the exception of spironolactone, these medications block ovarian function and prevent ovulation. GnRH analogs have also been used in the treatment of polycystic ovary syndrome because they suppress pituitary LH secretion and create a transitory and rapidly reversible condition of hypogonadotropic hypogonadism[15]; ovarian androgens are reduced and hirsutism improves during this treatment. Unfortunately, the profound hypoestrogenic state associated with GnRH analog administration prevents long-term (>6 months) treatment of polycystic ovary syndrome with this class of medications. At the suspension of GnRH analog administration, spontaneous menstrual cyclicity usually does not resume in patients with polycystic ovary syndrome who were anovulatory before GnRH analog treatment.[16]

Ovulation induction is often resorted to in women with polycystic ovary syndrome willing to conceive. Administration of clomiphene citrate results in an apparently high rate of ovulation but may be associated with luteal phase defects.[17] A discrepancy exists between the incidence of apparent ovulation and conception achieved with clomiphene; thus more effective means of ovulation induction are often needed. Human menopausal gonadotropins (hMG) and purified FSH may be used in patients with polycystic ovary syndrome who are resistant to clomiphene. Purified FSH has a theoretic advantage over hMG because it should correct the imbalance between LH and FSH typical of polycystic ovary syndrome in favor of the latter hormone. However, the use of purified FSH may not be associated with significant clinical advantages over hMG.[18]

The administration of hMG can result in a greater incidence of ovarian hyperstimulations and multiple pregnancies in polycystic ovary syndrome than in other ovulatory disorders. Wang and Gemzell[19] showed that patients with polycystic ovary syndrome are particularly prone to development of these complications. Therefore, ovulation induction with hMG should be monitored with particular care in patients with polycystic ovary syndrome.

Ovulation induction with pulsatile GnRH in polycystic ovary syndrome

Because of the low incidence of complications associated with the use of pulsatile GnRH, this therapeutic modality would appear to be optimal for the treatment of patients with polycystic ovary syndrome. Unfortunately, several reports have suggested that pulsatile GnRH may be far less effective in polycystic ovary syndrome than in hypogonadotropic hypogonadism.

Ory et al.[20] gave low-dose (25 ng/kg) pulsatile GnRH to four patients with polycystic ovary syndrome for six cycles. Ovulation apparently occurred in five cycles, but an inadequate luteal function may have been present in some subjects and no pregnancies ensued. Similarly, Burger et al.[21] reported a rather high ovulatory rate (87%) in 85 treatment units, whereas only five pregnancies occurred in 3 of the 11 patients treated.

An important distinction between patients with polycystic ovary syndrome and women with multifollicular ovaries was drawn by Adams et al.[22] Patients with multifollicular ovaries had lower LH and androgen levels and normal-size ovaries; these subjects responded well to exogenous pulsatile GnRH when administered both intravenously and subcutaneously, and an ovulatory rate per cycle of 85% was achieved. Conversely, patients with large polycystic ovaries and elevated serum LH and testosterone levels obtained only a 40% ovulatory

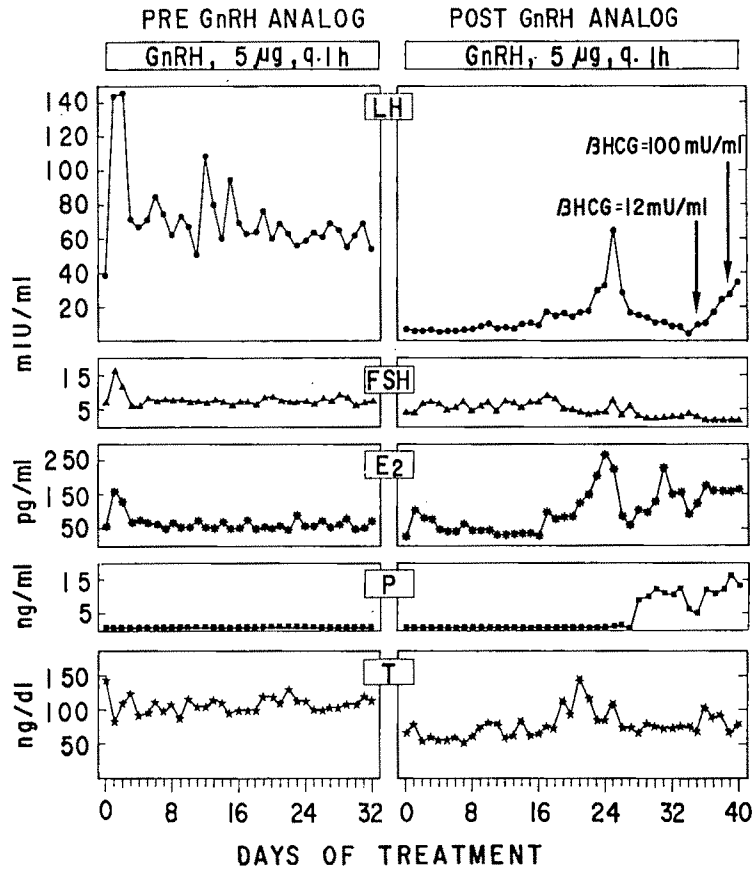


Fig. 2. Ovulation induction with pulsatile GnRH (5 μg/bolus every 60 minutes) in the same patient with polycystic ovary syndrome before *(left panels)* and after *(right panels)* GnRH analog suppression. (From Filicori M, et al. J Clin Endocrinol Metab 1988;66:327.)

rate per cycle; the success rate was similar in patients treated intravenously or subcutaneously. Thus patients with multifollicular ovaries seem to represent a different pathologic entity well differentiated from polycystic ovary syndrome and highly susceptible to pulsatile GnRH ovulation induction. These authors also found an elevated incidence of early miscarriage and abortion in polycystic ovary syndrome; only two of eight pregnancies were uneventful in these patients. The same group[23] later reported slightly better results in patients with polycystic ovary syndrome treated with a combination of subcutaneous pulsatile GnRH and clomiphene citrate; the intravenous route was resorted to in patients who failed to ovulate on the subcutaneous regimen. Nevertheless, only 87 (51%) of 170 treatment cycles were ovulatory, and conception was achieved in 23 cycles (13.5% pregnancy rate per cycle). The cumulative conception rate during a 6-month period was 60%. Lack of response was particularly evident in obese patients and subjects with higher testosterone levels. These investigators confirmed the occurrence of a high rate of spontaneous abortion in this patient population.

The administration of pulsatile GnRH for ovulation induction was found to be highly disappointing also by Hurwitz et al.[24] and Wilson et al.[25] The former group attained ovulation in only 2 of 12 treatment cycles, whereas the latter group had no success in 9 treatment periods of up to 6 weeks in 5 patients.

We recently confirmed[26] that the simple administration of intravenous pulsatile GnRH (5 μg every 60 minutes) in polycystic ovary syndrome results in low ovulatory (38% per cycle) and pregnancy rates (8% per cycle). However, a preliminary report by our group[4] suggested that pretreatment with a GnRH analog (buserelin, Hoechst-Roussel; 300 μg, subcutaneously, twice a day for 8 weeks) immediately before the initiation of pulsatile GnRH administration could improve the response of patients with polycystic ovary syndrome to this form of therapy (Fig. 2). When this approach was tested in a larger patient population of 23 patients with polycystic ovary syndrome,[26] we verified that a more physiologic endocrine pattern and higher ovulatory (90%) and pregnancy rates (38%) can be achieved with this regimen in polycystic ovary syndrome.

The pattern of hormonal secretion in patients with polycystic ovary syndrome simply stimulated with pul-

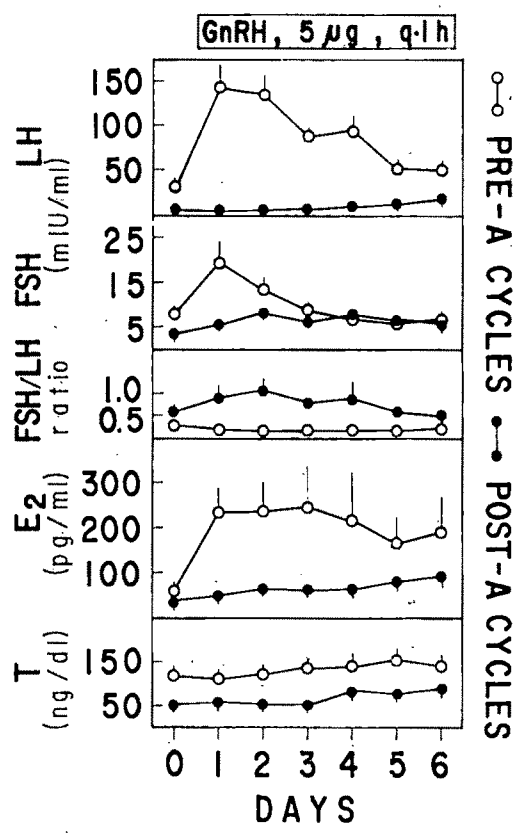


Fig. 3. Mean (±SE) serum gonadotropin and gonadal steroid levels in the early follicular phase (initial 6 days) of menstrual cycles induced with pulsatile GnRH in the same patients with polycystic ovary syndrome before *(pre-A)* and after *(post-A)* GnRH analog suppression. *T*, Testosterone; E_2, estradiol. (From Filicori M, et al. J Clin Endocrinol Metab 1988;66:327.)

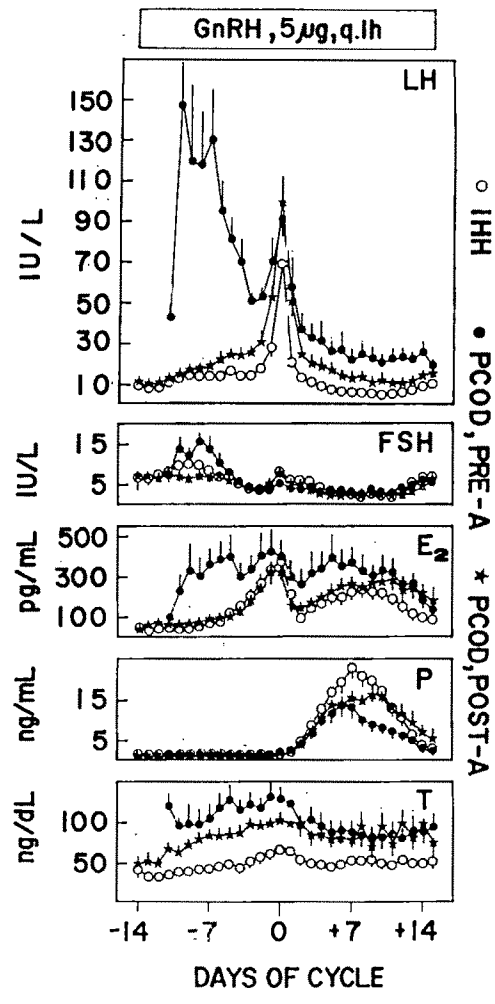


Fig. 4. Daily serum gonadotropin and gonadal steroid concentrations (mean ± SE) during pulsatile GnRH administration in ovulatory cycles of patients with primary hypogonadotropic amenorrhea *(IHH)* and polycystic ovarian disease *(PCOD)*, before *(pre-A)* and after *(post-A)* GnRH analog suppression. (From Filicori M, et al. J Clin Endocrinol Metab 1989;69:825.)

satile GnRH was characterized by high serum levels of LH, estradiol, and testosterone[26]; these features are indicative of an excessive pituitary and gonadal response in these patients. GnRH analog pretreatment in polycystic ovary syndrome induced a profound hypogonadotropic and hypogonadal state and completely abolished excessive gonadotropin and gonadal steroid secretion at the outset of pulsatile GnRH administration (Fig. 3).[4]

We then compared the endocrine response to pulsatile GnRH between patients with polycystic ovary syndrome and patients with primary amenorrhea caused by hypogonadotropic hypogonadism.[26] Pulsatile GnRH administration in this form of profound hypogonadotropic hypogonadism reproduced the pattern of reproductive hormones encountered in the normal menstrual cycle. In the cycles done after GnRH analog suppression, the profile of LH secretion closely resembled the one of patients with hypogonadotropic hypogonadism although mean LH levels were slightly more elevated (Fig. 4). Serum estradiol levels were practically superimposable in cycles done after GnRH analog suppression of patients with polycystic ovary syndrome and hypogonadotropic hypogonadism and comparable to normal levels, suggesting more physiologic folliculogenesis; progesterone levels were higher in cycles done after GnRH analog suppression than in preanalog cycles, indicating better corpus luteum function. Finally, although follicular-phase testosterone levels were lower in cycles done after GnRH analog suppression than in preanalog cycles in polycystic ovary syndrome, such differences disappeared in the luteal phase. This latter finding suggests that part of the benefits of GnRH analog suppression may be lost by 3 to 4 weeks after GnRH analog suppression. In fact, when pulsatile GnRH was continued for a second consecutive

cycle done after GnRH analog suppression, a lower ovulatory rate was obtained.

Obesity was another factor that seemed to affect the outcome of pulsatile GnRH therapy negatively in our patients. Only 15% of preanalog cycles in obese patients with polycystic ovary syndrome were ovulatory compared with 64% in nonobese subjects. GnRH analog pretreatment improved response in both groups but, although all cycles done after GnRH analog suppression were ovulatory in nonobese subjects, 25% of obese patients still did not respond to pulsatile GnRH.

Spontaneous abortion also appeared to be a conspicuous complication among our patients with polycystic ovary syndrome because it occurred in 50% of preanalog cycles and 38% of cycles done after GnRH analog suppression. However, our data suggest that miscarriage in polycystic ovary syndrome should not be related directly to high LH levels in the follicular phase,[27] because this complication also was prominent in cycles done after GnRH analog suppression that were characterized by reduced serum LH concentrations.

In conclusion, pulsatile GnRH appears to be effective in polycystic ovary syndrome only when these patients are given pretreatment and transiently rendered hypogonadotropic with a GnRH analog. By lowering pituitary LH secretion and ovarian androgen levels, this approach leads to a temporary condition of hypogonadotropic hypogonadism that creates a window of enhanced susceptibility of patients with polycystic ovary syndrome to pulsatile GnRH ovulation induction. However, to be maximally effective, GnRH analog administration should probably precede each pulsatile GnRH ovulation induction attempt because the advantages of pituitary suppression are rapidly lost. Obesity is associated with lower success rates, and spontaneous abortion remains an important complication in polycystic ovary syndrome even after GnRH analog suppression. These findings should extend the applicability of pulsatile GnRH and lead to high ovulatory and pregnancy rates and greatly reduced risks of ovarian hyperstimulation and multiple pregnancy in the great majority of patients with anovulatory disorders not associated with primary hypogonadism.

REFERENCES

1. Louvet JP, Mitchell HS, Schreiber JR, Ross GT. Evidence for a role of androgens in follicular maturation. Endocrinology 1975;97:366.
2. Loughlin T, Cunningham S, Moore A, Culliton M, Smyth PPA, McKenna TJ. Adrenal abnormalities in polycystic ovary syndrome J Clin Endocrinol Metab 1986;62: 142.
3. Waldstreicher J, Santoro N, Hall JE, Filicori M, Crowley WF Jr. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotrope desensitization. J Clin Endocrinol Metab 1988;66:165.
4. Filicori M, Campaniello E, Michelacci L, et al. Gonadotropin-releasing hormone (GnRH) analog suppression renders polycystic ovarian disease patients more susceptible to ovulation induction with pulsatile GnRH. J Clin Endocrinol Metab 1988;66:327.
5. Kazer RR, Kessel B, Yen SSC. Circulating luteinizing hormone pulse frequency in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1987;65:233.
6. Venturoli S, Porcu E, Fabbri R, et al. Episodic pulsatile secretion of FSH, LH, prolactin, oestradiol, oestrone, and LH circadian variations in polycystic ovary syndrome. Clin Endocrinol (Oxf) 1988;27:1.
7. Burger CW, Korsen T, van Kessel H, Van Dop PA, Caron FJM, Schoemaker J. Pulsatile luteinizing hormone patterns in the follicular phase of the menstrual cycle, polycystic ovarian disease (PCOD) and non-PCOD secondary amenorrhea. J Clin Endocrinol Metab 1985;61:1126.
8. Rebar R, Judd HL, Yen SSC, Rakoff J, Vandberg G, Naftolin F. Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest 1976;57:1320.
9. Lobo RA, Granger L, Goebelsman U, Mishell DR. Elevations in unbound serum estradiol as a possible mechanism for inappropriate gonadotropin secretion in women with PCO. J Clin Endocrinol Metab 1981;52:156.
10. Poretsky L, Kalin MF. The gonadotropin function of insulin. Endocrin Rev 1987;8:132.
11. Pasquali A, Antenucci D, Casimirri F, et al. Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab 1989;68:173.
12. Raj SG, Raj MHG, Talbert LM, Sloan CS, Hicks B. Normalization of testosterone levels using a low estrogen–containing oral contraceptive in women with polycystic ovary syndrome. Obstet Gynecol 1982;60:15.
13. Hammerstein J, Meckies J, Leo-Rossberg I, Motz L, Zielske F. Use of cyproterone acetate (CPA) in the treatment of acne, hirsutism, and virilism. J Steroid Biochem 1975;6:827.
14. Cumming DC, Yang JC, Rebar RW, Yen SSC. Treatment of hirsutism with spironolactone. JAMA 1982;247:1295.
15. Couzinet B, Le Strat N, Brailly S, Schaison G. Comparative effects of cyproterone acetate or a long-acting gonadotropin-releasing hormone agonist in polycystic ovarian disease. J Clin Endocrinol Metab 1986;63:1031.
16. De Ziegler D, Steingold K, Cedars M, et al. Recovery of hormone secretion after chronic gonadotropin-releasing hormone agonist administration in women with polycystic ovarian disease. J Clin Endocrinol Metab 1989;68:1111.
17. Yen SSC, Vela P, Ryan KJ. Effect of clomiphene citrate in polycystic ovary syndrome: relationship between serum gonadotropin and corpus luteum function. J Clin Endocr 1970;31:7.
18. Venturoli S, Paradisi R, Fabbri R, Porcu E, Orsini LF, Flamigni C. Induction of ovulation in polycystic ovary: human menopausal gonadotropin or human urinary follicle stimulating hormone? Int J Fertil 1987;32:66.
19. Wang CF, Gemzell C. The use of human gonadotropins for the induction of ovulation in women with polycystic ovarian disease. Fertil Steril 1980;33:479.
20. Ory SJ, London SN, Tyrey L, Hammond CB. Ovulation induction with pulsatile gonadotropin-releasing hormone administration in patients with polycystic ovarian syndrome. Fertil Steril 1985;43:20.
21. Burger CW, Korsen TJ, Hompes PG, van Kessel H, Schoemaker J. Ovulation induction with pulsatile luteinizing releasing hormone in women with clomiphene citrate–resistant polycystic ovary-like disease: clinical results. Fertil Steril 1986;46:1045.
22. Adams J, Frank S, Polson DW, et al. Multifollicular ova-

ries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. Lancet 1985;2:1375.
23. Eshel A, Abdulwahid NA, Armar NA, Adams JM, Jacobs HS. Pulsatile luteinizing hormone–releasing hormone therapy in women with polycystic ovary syndrome. Fertil Steril 1988;49:956.
24. Hurwitz A, Rosenn B, Palti Z, Ebstein B, Har Nir R, Ron M. The hormonal response of patients with polycystic ovarian disease to subcutaneous low-frequency pulsatile administration of luteinizing hormone-releasing hormone. Fertil Steril 1986;46:378.
25. Wilson JM, Traub AI, Sheridan B, Thompson W, Atkinson AB. Conventional dose intravenous pulsatile GnRH therapy does not induce ovulation in polycystic ovarian disease. Acta Endocrinol (Copenh) 1988;117:289.
26. Filicori M, Flamigni C, Campaniello E, et al. The abnormal response of polycystic ovarian disease patients to exogenous pulsatile gonadotropin-releasing hormone: characterization and management. J Clin Endocrinol Metab 1989;69:825.
27. Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS. Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. Br Med J 1988;297:1024.

Discussion

Audience. In your studies, how did you define polycystic ovary syndrome?

Dr. Filicori. If you look in the literature, you find that the definition of this disorder is heterogeneous. We insisted that both LH and androgen levels be elevated in these patients because we were really trying to approach the problem of LH-dependent hyperandrogenism. So all the patients I have shown have both derangements.

Audience. Do you have any explanation for the increased abortion rate in patients with polycystic ovary syndrome after ovulation induction?

Dr. Filicori. We have not closely monitored these pregnancies, but I can give you our thoughts on it. We had thought that this problem could be related to an abnormal endometrium. We had hoped that by lowering both gonadotropin and gonadal steroids with the GnRH analog and thus reducing endometrial hypertrophy, we could improve on the high abortion rate we had observed in the preanalog cycles of ovulation induction. Unfortunately, the abortion rate also remained high in the postanalog cycles. This problem is probably not related to high follicular phase LH levels because, after analog suppression in these women, LH levels are very close to normal. So we really have no idea what causes this high abortion rate. In general, a high abortion rate is also present when other methods of ovulation induction are applied to patients with polycystic ovary syndrome.

Audience. Have you looked at androgen levels in the luteal phase in cycles with and without analog suppression?

Dr. Filicori. By the end of the follicular phase and throughout the luteal phase of postanalog cycles, androgen concentrations return to the preanalog levels.

Audience. You set your dose at 5 μg. Do you think that you might have a different response if you adjusted the dose on a nanogram per kilogram basis?

Dr. Filicori. We selected this dose because it is relatively close to the physiologic dose of 75 ng/kg, as reported by Drs. Santoro and Crowley. After analog suppression, the dose of GnRH is not very relevant because you usually get a slightly long follicular phase but no sign of excessive ovarian stimulation. You could try higher doses, but you may run into other problems. In hypogonadotropic hypogonadism, we have achieved good ovulatory response and pregnancy rates, with lower multiple conception rates, with the lower dose of 2.5 μg per bolus.

Audience. How often have you seen hyperstimulation in the stimulatory phase of analog treatment?

Dr. Filicori. We have never had any problem of excessive ovarian stimulation with analog administration. You may have some ovarian enlargement, but it will not require specific monitoring and usually regresses when pituitary suppression is achieved.

The luteinizing hormone–releasing hormone pulse generator in men: Abnormalities and clinical management

Alvin M. Matsumoto, MD, Kenneth M. Gross, MD, Carol B. Sheckter, MD, and William J. Bremner, MD, PhD

Seattle, Washington

Most men with hypogonadotropic eunuchoidism have absent luteinizing hormone and presumably absent luteinizing hormone–releasing hormone pulses. Pulsatile luteinizing hormone–releasing hormone therapy is effective in restoring normal gonadotropin secretion and testicular function and inducing fertility in men with hypogonadotropic eunuchoidism. Furthermore, pulsatile (versus continuous) luteinizing hormone–releasing stimulation of the pituitary gland is an absolute requirement for normal gonadotropin secretion. Men with idiopathic oligoazoospermia and selective elevation of follicle-stimulating hormone levels have slow luteinizing hormone and presumably luteinizing hormone–releasing pulse frequency. In these men, pulsatile luteinizing hormone–releasing treatment is effective in decreasing serum follicle-stimulating hormone levels, but it is unclear whether spermatogenesis and fertility are improved. (AM J OBSTET GYNECOL 1990;163:1743-52.)

Key words: Hypogonadotropic eunuchoidism, luteinizing hormone, luteinizing hormone–releasing hormone, idiopathic oligoazoospermia, follicle-stimulating hormone

Secretion of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) by the anterior pituitary gland is controlled by LH-releasing hormone (LHRH) secretion from the hypothalamus. LHRH is released episodically into the hypophyseal portal system and stimulates the pulsatile secretion of LH and FSH into the peripheral circulation, which in turn regulate gonadal function.

Because of its short circulating half-life,[1] LHRH is found in highest concentrations in the hypophyseal portal circulation and is difficult to detect in peripheral blood, making direct clinical assessment of pulsatile LHRH secretion impossible. Studies in experimental animals have confirmed that the pattern of pulsatile LH secretion into peripheral blood is a very good index of episodic LHRH release from the hypothalamus.[2, 3] Therefore we and others have used frequent blood-sampling studies to characterize LH pulse patterns carefully as indexes of hypothalamic LHRH pulse-generator activity in humans.[4, 5]

Two major disorders of LHRH pulse generation have been identified by comparing the patterns of pulsatile LH secretion in these disorders with those of normal men: (1) hypogonadotropic eunuchoidism or idiopathic hypogonadotropic hypogonadism and (2) idiopathic oligoazoospermia with selectively elevated serum FSH levels.

From the Gerontology Research, Education, and Clinical Center and Medical Service, Veterans Administration Medical Center, and the Population Center for Research in Reproduction, Divisions of Gerontology and Geriatric Medicine and Metabolism, Endocrinology, and Nutrition, Department of Medicine, University of Washington School of Medicine.

Supported by National Institutes of Health grant P50-HD-12629, Clinical Research Center grant RR-37, and Veterans Affairs Medical Research Funds.

Reprint requests: Alvin M. Matsumoto, MD, VA Medical Center, GRECC (182B), 1660 S. Columbian Way, Seattle, WA 98108.

6/0/24566

LHRH pulse generator in hypogonadotropic eunuchoidism

Hypogonadotropic eunuchoidism is a heterogeneous disorder characterized by failure to undergo normal pubertal development (usually by 18 years of age), low serum testosterone levels, low or normal serum gonadotropin concentrations, and otherwise normal anterior pituitary functioning.[6-8]

The clinical presentation of hypogonadotropic eunuchoidism is variable because of differences in the onset and severity of the disorder. The hypogonadotropic hypogonadism is often associated with anosmia or hyposomia (Kallmann's syndrome) and other midline morphologic and functional defects such as cleft lip and palate.[6, 8] The mode of inheritance of hypogonadotropic eunuchoidism is variable.[6, 7] The underlying pathophysiology of the hypogonadotropic hypogonadism is presumed to be relative deficiency of LHRH secretion.

To characterize the abnormalities in the LHRH pulse generator in men with hypogonadotropic eunuchoidism, frequent blood-sampling studies (e.g., every 10 minutes for 24 hours) have been performed to define the secretory patterns of hypogonadotropic eunuchoidism, which reflect those of LHRH.[5, 9] Compared with

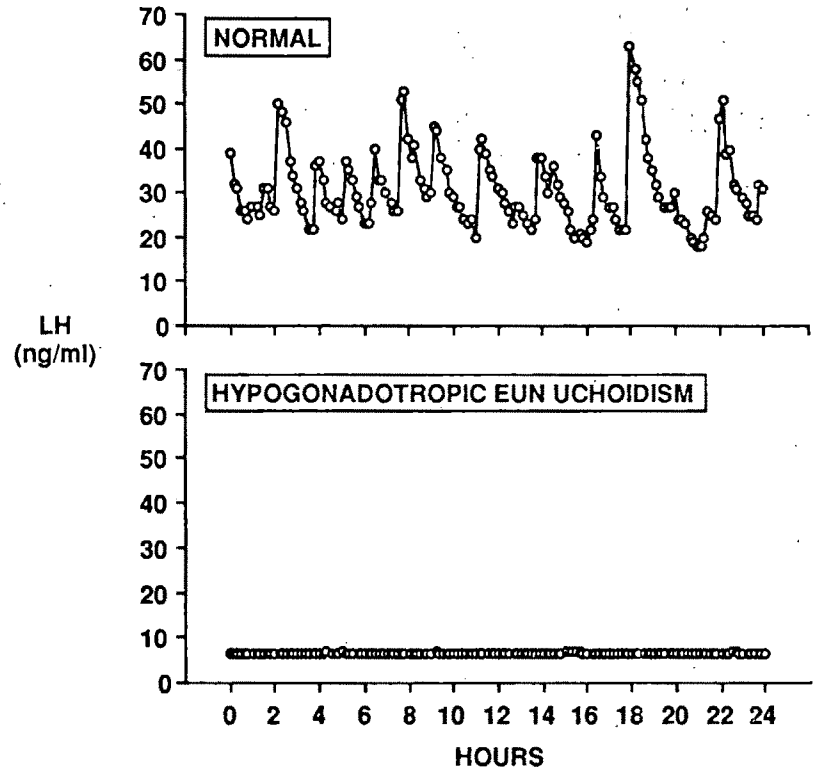


Fig. 1. LH pulse patterns in a normal man *(top)* and a man with hypogonadotropic eunuchoidism *(bottom)*, as determined on frequent blood sampling studies performed every 10 minutes for 24 hours. Note the absence of spontaneous LH pulses in the man with hypogonadotropic eunuchoidism.

normal men who demonstrate LH pulses every 100 to 120 minutes, the vast majority (more than 90%) of men with hypogonadotropic eunuchoidism have no detectable LH pulses during frequent blood sampling (i.e., an apulsatile pattern[5, 9]) (Fig. 1), suggesting absent or severely reduced LHRH pulse generation. In other men with hypogonadotropic eunuchoidism, decreases in LH pulse amplitude or frequency have been reported,[5, 9] suggesting disordered LHRH pulse generation.

LHRH pulse generator in oligoazoospermia with selective elevation of FSH levels

Approximately 5% to 6% of men in the reproductive age group are infertile. The majority of infertile men have disordered spermatogenesis of unknown cause (i.e., idiopathic oligoazoospermia). Approximately 20% of men with oligoazoospermia have elevated serum FSH and normal serum LH and testosterone levels.[10]

Several mechanisms have been proposed to explain the selective elevations in FSH relative to LH levels in these men, including increased pituitary stimulation by a separate FSH-releasing factor and decreased testicular production of negative feedback factors, such as inhibin[11] and/or sex steroids.[12] We and others have found evidence for an alternative hypothesis to explain selective elevation of FSH levels (i.e., that the frequency of LHRH stimulation of the pituitary gland is capable of differentially regulating FSH and LH levels).[13, 14]

Men with selectively increased FSH levels often demonstrate a reduced LH pulse frequency compared with normal men (Fig. 2). We performed frequent blood sampling every 10 minutes for 24 hours to characterize LH pulse patterns in a group of five men with elevated FSH and normal LH and testosterone levels and seven age-matched normal men. Men with selective elevations in FSH levels had fewer LH pulses per 24 hours (10.6 ± 0.5, mean ± SEM) than had normal men (12.9 ± 0.6; $p < 0.01$), with no significant differences in LH pulse amplitude and total and free testosterone and estradiol levels.[13] Other investigators have also found a reduced LH pulse frequency in men with selective elevations in FSH levels compared with normal men.[14] These findings suggest that the frequency of LHRH pulse generation is reduced in men with idiopathic oligoazoospermia and selective elevation in FSH levels.

The spermatogenic defect in men with idiopathic oligoazoospermia has been attributed to a primary testicular disorder. However, there is generally no history of known causes of testicular damage, and biopsy speci-

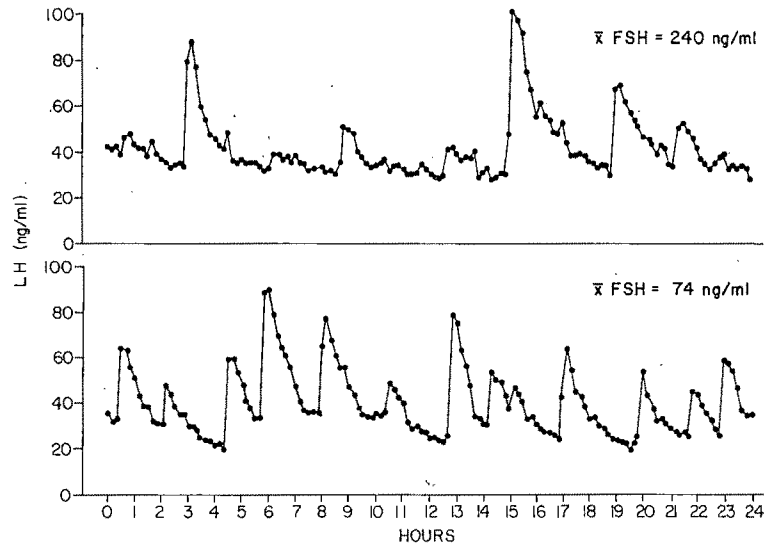


Fig. 2. LH pulse patterns in a man with idiopathic oligoazoospermia and selective elevation of serum FSH levels *(top)* and a normal man *(bottom)*, as determined on frequent blood-sampling studies performed every 10 minutes for 24 hours. Note the decreased frequency of spontaneous LH pulses in the man with oligoazoospermia and selective elevations of FSH levels.

mens of testes do not demonstrate pathologic correlates of injury, such as inflammation or fibrosis.[10] Therefore it is possible that some men with idiopathic oligoazoospermia may have a primary hypothalamic rather than testicular disorder, resulting in decreased LHRH pulse frequency, selective elevations in FSH levels, and disordered spermatogenesis as a result of the altered hormonal milieu. We and others are beginning to test this hypothesis by determining the effect of long-term pulsatile LHRH administration at increased pulse frequencies on sperm production in men with idiopathic oligoazoospermia and selective elevations in FSH levels (see below).

Treatment of hypogonadotropic eunuchoidism

Most men with hypogonadotropic eunuchoidism have deficient hypothalamic LHRH pulse generation, resulting in absent pulsatile gonadotropin secretion and lack of spontaneous pubertal development. Depending on the clinical situation, hypogonadotropic eunuchoidism may be treated with testosterone, gonadotropins, or "physiologic" pulsatile LHRH replacement.

If pubertal development and normal sexual functioning are desired but induction of fertility is not an issue, treatment of hypogonadotropic eunuchoidism is most practically accomplished with testosterone therapy. A long-acting 17β-hydroxyl ester of testosterone, such as testosterone enanthate or cypionate, 100 to 200 mg intramuscularly every 10 days to 2 weeks, results in significant virilization by 4 to 6 months of treatment.[15] The development of longer-acting analogs of testosterone may reduce the requirement for injections to every 3 to 4 months.

When stimulation of sperm production and induction of fertility are desired, gonadotropin or pulsatile LHRH therapy is required. Gonadotropin therapy is usually initiated with a preparation containing LH-like activity alone, such as human chorionic gonadotropin (hCG), 1000 to 3000 units subcutaneously or intramuscularly two to three times weekly for 6 months, to stimulate testicular steroidogenesis and induce virilization.[16-19] In some men, treatment with hCG alone may also induce spermatogenesis. However, in most men with hypogonadotropic eunuchoidism a preparation with FSH activity, such as human menopausal gonadotropin, 37.5 to 150 units subcutaneously or intramuscularly two to three times weekly, must be coadministered with hCG for another 6 months to 1 year to stimulate sperm production and induce fertility.[16-19] Prior testosterone therapy does not alter subsequent testicular responsiveness to gonadotropin therapy.[18, 20]

With the availability of LHRH for clinical use and portable, automatic infusion pumps, long-term, physiologic LHRH-replacement therapy for hypogonadotropic eunuchoidism has become a practical therapeutic option for men with hypogonadotropic eunuchoidism. In general, low doses (5 to 20 μg) of subcutaneous LHRH are administered at a frequency similar to that in normal men (i.e., every 2 hours).[5, 21] Pulsatile LHRH administration in men with hypogonadotropic eunuchoidism results in a rapid restoration of pituitary gonadotropin secretion by several weeks of treatment (Fig. 3) and normalization of testosterone secretion during the initial 3 months of therapy.[22] Continued pulsatile LHRH treatment for several months or years can stimulate sperm production and induce fer-

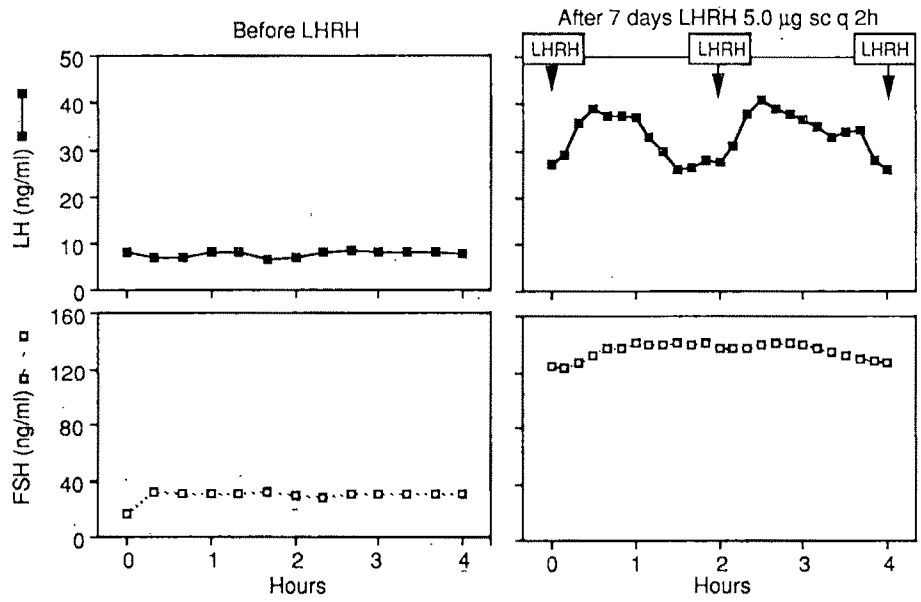


Fig. 3. Mean serum LH *(top)* and FSH *(bottom)* levels during frequent blood sampling every 20 minutes for 4 hours in four men with hypogonadotropic eunuchoidism before *(left)* and after 7 days of pulsatile LHRH therapy (5 μg subcutaneously every 2 hours). LH and FSH levels are low and there are no spontaneous LH pulsations before LHRH therapy. After 7 days of pulsatile LHRH treatment, both LH and FSH levels increase into the normal adult male range (8 to 60 ng/ml and 30 to 230 mg/ml, respectively), and discrete LH pulses follow each LHRH bolus, mimicking a normal physiologic LH pulse pattern.

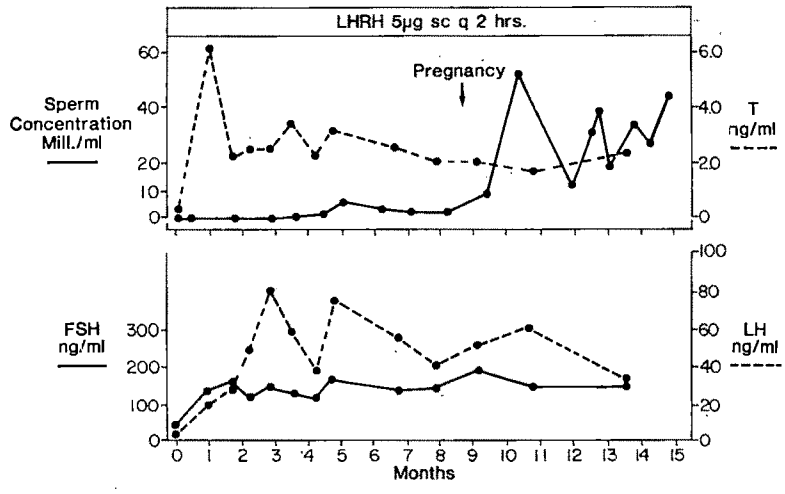


Fig. 4. Testicular *(top)* and gonadotropin *(bottom)* responses to long-term pulsatile LHRH therapy (5 μg every 2 hours for 15 months) in a man with hypogonadotropic eunuchoidism. Pulsatile LHRH treatment increases LH, FSH, and testosterone *(T)* levels into the normal adult male range by 1 month and stimulates sperm production by 9 months, resulting in fertility. (From Matsumoto AM, Bremner WJ. Bailliere's Clin Endocrinol Metab 1987;1:71.)

tility in men with hypogonadotropic eunuchoidism (Fig. 4).[21,22] The ability of exogenous pulsatile LHRH administration to restore pituitary gonadotropin and testicular function is consistent with the hypothesis that hypogonadotropic eunuchoidism is caused by a deficiency in LHRH pulse generation.

Previous studies in hypothalamic-lesioned, LHRH-deficient monkeys have demonstrated that a pulsatile mode of LHRH administration is absolutely necessary for normal pituitary gonadotropin secretion, whereas continuous administration failed to maintain normal gonadotropin levels.[23] In humans continuous

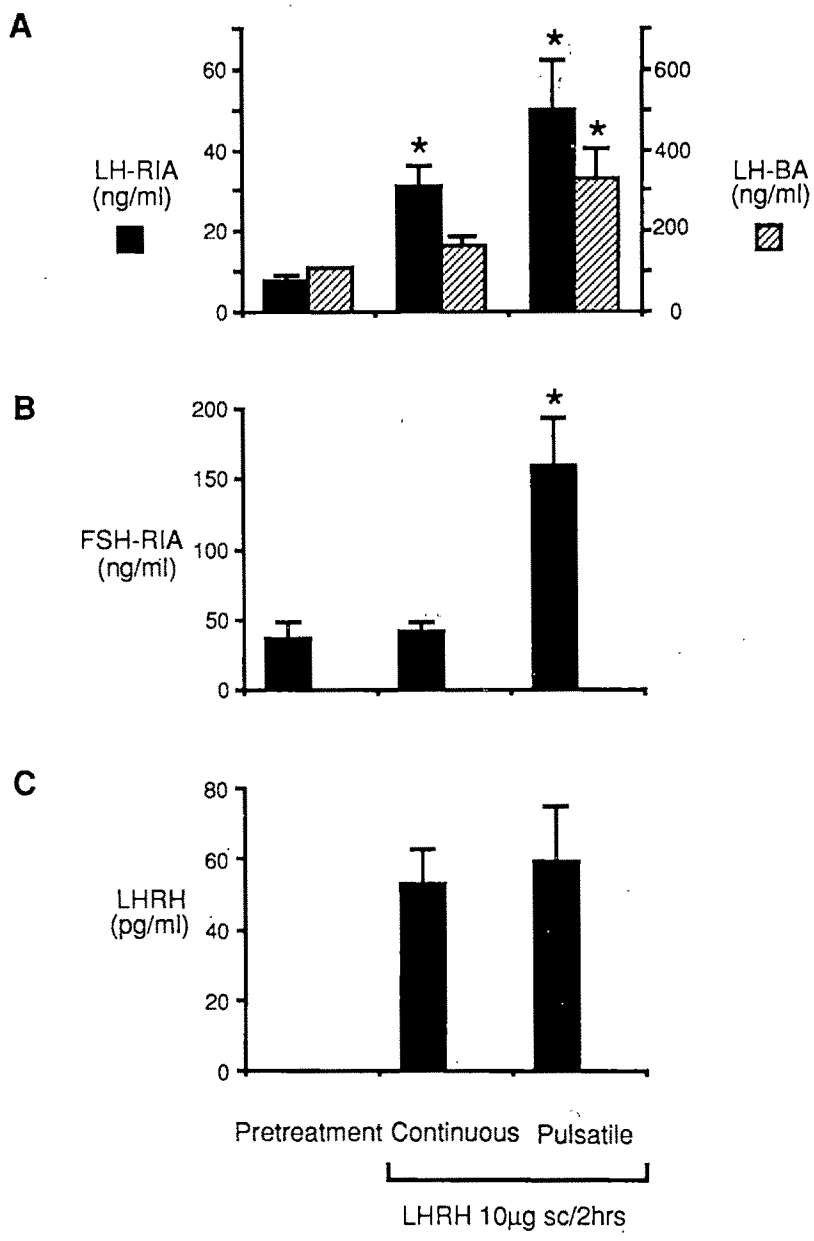


Fig. 5. Mean (±SEM) serum LH by radioimmunoassay *(RIA)* and bioassay *(BA)*, FSH, RIA, and LHRH levels in four men with hypogonadotropic eunuchoidism before (pretreatment) and at the end of 1 week each of continuous and pulsatile LHRH infusions (10 μg every 2 hours). Compared with pretreatment values, serum LH-RIA levels *(solid bars, A)* increased significantly during both continuous and pulsatile LHRH treatment. However, compared with pretreatment levels, LH-BA *(hatched bars, A)* and FSH-RIA *(B)* were not significantly different during the continuous regimen but did increase significantly during pulsatile LHRH administration. LHRH concentrations *(C)* were similar during the continuous and pulsatile regimens. $*p < 0.05$, compared with pretreatment values. (From Matsumoto AM, Gross KM, Bremner WJ. The physiological significance of pulsatile LHRH secretion in man: gonadotropin responses to physiological doses of pulsatile versus continuous LHRH administration. Int J Androl [in press]. Copyright 1990 by Scriptor Publisher ApS, Copenhagen.)

administration of markedly supraphysiologic LHRH doses[24, 25] or treatment with very potent, long-acting LHRH agonistic analogs[26] initially stimulates and then suppresses LH and FSH secretion. However, it was not known whether continuous administration of lower, more physiologic doses of LHRH (i.e., dosages known to be effective in restoring testicular function when given in a pulsatile manner) could stimulate normal pituitary gonadotropin secretion in humans.

To assess the physiologic significance of pulsatile LHRH stimulation of the pituitary gland, we compared the gonadotropin responses to a physiologic dose of

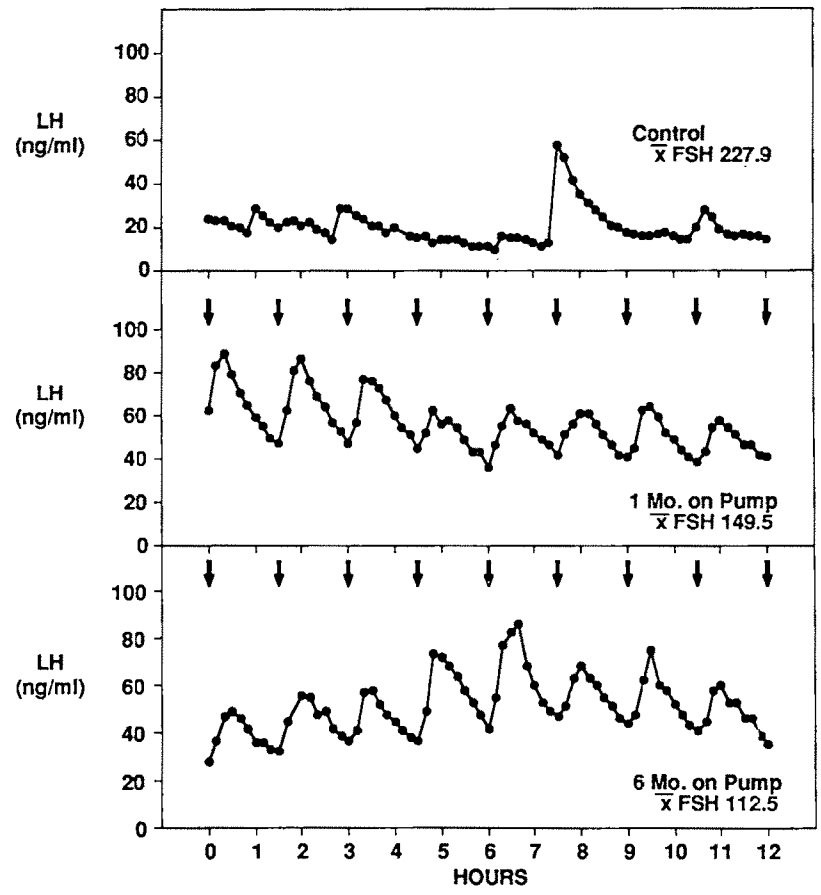


Fig. 6. Serum LH levels during frequent blood sampling every 10 minutes for 12 hours in a man with idiopathic oligoazoospermia and selective elevation of FSH levels before (control) and 1 and 6 months after pulsatile LHRH therapy (5 μg every 90 minutes). *Arrows* indicate the timing of LHRH pulses delivered by an automatic, portable infusion pump. Note that long-term pulsatile LHRH treatment increased the LH pulse frequency and mean LH levels while decreasing mean FSH levels.

LHRH administered in pulsatile versus continuous pattern in four men with hypogonadotropic eunuchoidism.[27] These men stopped hormone treatment for 5 to 6 weeks before being studied (pretreatment) and then were treated with LHRH, 5 μg subcutaneously every 2 hours for 1 week, to prime pituitary responsiveness. After this period of LHRH priming, each man received the same physiologic dose (10 μg) subcutaneously every 2 hours in a pulsatile fashion for 1 week and as a continuous infusion for 1 week, in varying order. Frequent blood sampling for LH, FSH, and LHRH was performed every 20 minutes for 4 hours during the pretreatment period and at the end of each LHRH regimen. We found that pulsatile LHRH administration in a physiologic dose stimulated normal pituitary LH and FSH secretion (Fig. 5). In contrast, continuous LHRH infusion of the same physiologic LHRH dose increased immunoreactive LH levels but did not stimulate bioactive LH levels or FSH levels above pretreatment values (Fig. 5). Serum LHRH (Fig. 5), testosterone, and estradiol levels were comparable during the pulsatile and continuous infusions. These results demonstrate that a pulsatile versus continuous LHRH signal is an absolute requirement for normal pituitary gonadotropin secretion in men. Therefore, they suggest that low-dose continuous LHRH administration is unlikely to stimulate normal testicular function.

Treatment of men with oligoazoospermia who demonstrate selective elevations of FSH levels

Infertile men with idiopathic oligoazoospermia and high serum FSH levels are considered to have irreversible seminiferous tubular damage. Many therapeutic modalities have been tried but have been uniformly unsuccessful in improving sperm production and fertility in these men. However, compared with normal men, men with idiopathic oligoazoospermia and selective elevation of FSH levels demonstrate a decreased frequency of spontaneous LH pulses. This raises the possibility that some of these men may have a primary abnormality of hypothalamic LHRH pulse generation. We hypothesized that the disordered spermatogenesis

in these men may be a consequence of the altered hormonal milieu induced by the abnormally slow LHRH pulse frequency, and LHRH administration at more rapid pulse frequencies may reduce FSH levels and improve sperm production.

As an initial test of this hypothesis, we have shown that short-term administration of LHRH at increasing pulse frequencies results in a progressive decrease in serum FSH levels into the normal range, without significant changes in serum LH, testosterone, and estradiol levels.[28] Similar findings have been reported by others.[29]

The effects of LHRH on LH and FSH levels in our study are consistent with previous work in animals[30-32] and men,[13, 28, 29, 33] which has shown that increasing the LHRH pulse frequency leads to more rapid LH pulses but lower mean FSH levels. Conversely, slowing the LHRH pulse frequency led to slower LH pulses and higher FSH levels. This finding of a differential effect of LHRH pulse frequency on LH and FSH levels has been extended by more recent work in animals demonstrating selective control of gonadotropin subunit gene expression by the frequency of pulsatile LHRH stimulation.[34]

To test further the hypothesis that reduced LHRH pulse frequency may cause or contribute to the spermatogenetic defect in men with idiopathic oligoazoospermia and selective elevation of FSH levels, we tested the effects of more prolonged (6 to 12 months) administration of pulsatile LHRH at a relatively rapid pulse frequency.

In this preliminary study we studied seven men with idiopathic oligoazoospermia (with mean sperm concentrations on three to six specimens during 3 months of less than 20 million/ml), elevated FSH levels (normal range 30 to 230 ng/ml), and normal LH and testosterone levels (8 to 60 ng/ml and 2.8 to 10.0 ng/ml, respectively). All subjects were infertile for more than 1 year but were otherwise healthy as determined by history and physical examination. None had known primary testicular disease and testis size ranged from 15 to 25 ml.

After a 3-month control period during which no hormones were administered, subjects began a 6-month treatment period throughout which they received LHRH, 5 μg subcutaneously every 90 minutes, into the abdominal wall by a portable automatic infusion pump (Zyklomat; Ferring Laboratories, Suffern, N.Y.). In two men pulsatile LHRH treatment at the same dosage was continued for 12 months. At the end of the treatment period, LHRH was discontinued and subjects were followed up for an additional 3 months (recovery period).

Throughout the entire study, seminal fluid analyses[35, 36] were performed every 2 weeks on semen samples obtained by masturbation after 48 hours of abstinence from ejaculation. Frequent blood sampling for LH and FSH levels by radioimmunoassay[4, 36] was performed every 10 minutes for 12 hours at the end of the control period, after 1 and 6 months of treatment, and at the end of the recovery period; testosterone and estradiol levels were measured by radioimmunoassay[37] in single samples at the beginning of the frequent sampling studies (7:00 to 9:00 AM).

During the control period, all subjects demonstrated a slow spontaneous LH pulse frequency typical of this group of patients.[13] After 1 and 6 months of LHRH treatment, 5 μg every 90 minutes, LH pulse frequency increased and the LH patterns in all men were directly related to the activity of the LHRH pump (Fig. 6). After exogenous LHRH was discontinued, LH pulse frequency reverted to the typically slower frequency exhibited in the control period.

Compared with control levels (41 ± 4 ng/ml), LH levels increased significantly after 1 and 6 months of LHRH treatment (70 ± 7 and 70 ± 13 ng/ml, respectively; both $p < 0.05$). Three months after discontinuation of LHRH, LH levels decreased to 35 ± 5 ng/ml and were comparable to control values. During the control period, FSH levels (311 ± 25 ng/ml) were considerably higher than the upper limit of the normal range for men. FSH levels decreased after 1 and 6 months of LHRH treatment to 236 ± 30 and 264 ± 52 ng/ml, respectively (borderline significance, $p < 0.09$), and rose to near control values (300 ± 42 ng/ml) in the recovery period.

Testosterone levels increased from 3.9 ± 0.2 ng/ml during the control period to 4.6 ± 0.7 and 5.4 ± 0.7 ng/ml after 1 and 6 months of LHRH treatment, respectively. Three months after LHRH therapy was completed, testosterone levels decreased to 3.3 ± 0.3 ng/ml. During the control period, estradiol levels were 26 ± 4 pg/ml, rose to 47 ± 5 pg/ml and 52 ± 9 pg/ml after 1 and 6 months of LHRH treatment, respectively, and then decreased to 35 ± 9 pg/ml at the end of the recovery period.

Before the LHRH treatment, five of seven men had azoospermia. No sperm appeared in the ejaculates of the subjects with azoospermia at any time during the study period. One man with control sperm counts of 0.6 to 7.0 million/ml remained severely oligospermic and infertile during LHRH therapy. The final subject had less severe oligospermia at baseline (sperm counts 2.0 to 26.2 million/ml). Although sustained improvement in his sperm counts during pulsatile LHRH treatment (2.8 to 37.4 million/ml) could not be established, this man impregnated his wife after 3 months of LHRH therapy. In two men, continuing LHRH therapy for as long as 12 months did not improve sperm production.

Our preliminary results demonstrate that the long-term administration of exogenous LHRH to men with

idiopathic oligoazoospermia with a more rapid physiologic pulse frequency can reliably increase their LH pulse frequency and decrease their mean serum FSH levels, but we were unable in these studies to demonstrate an improvement in sperm production.

Recently Bals-Pratsch et al.[38] reported results of long-term pulsatile LHRH therapy in nine men with idiopathic oligoazoospermia with elevated serum FSH levels that were similar to our findings. Six men with severe oligospermia (sperm counts <5 million/ml) and three men with azoospermia received the same LHRH dosage regimen that we used in our study (i.e., 5 μg subcutaneously every 90 minutes) by a portable infusion pump for 24 weeks. Despite lowering serum FSH levels, pulsatile LHRH treatment did not result in any improvement of sperm count or other seminal fluid parameters.

However, other groups have reported a stimulatory effect of long-term pulsatile LHRH treatment in men with oligospermia with elevated FSH levels. Wagner and von zur Mühlen[39] found that in some men with severe oligospermia (sperm counts <10 million/ml) and elevated serum FSH levels, pulsatile LHRH therapy (4 μg every 129 minutes) for 6 to 12 months increased progressively motile sperm density.

Recently Aulitzky et al.[40] determined the effect of long-term pulsatile LHRH treatment in men with varying degrees of oligospermia, elevated serum FSH levels, and slow spontaneous LH pulse frequency. Subjects were grouped according to the severity of their spermatogenic defect, based on the SPT score (number of type A_d *s*permatogonia *p*er *t*ubule square) and sperm density. Group 1, with the least testicular damage, had SPT scores of 1.0 or greater and sperm densities of 1 to 20 (8.84 ± 4.0) million/ml, group 2 had SPT scores of 0.25 to 1.0 and sperm densities of 1 to 20 (3.38 ± 1.75) million/ml, and group 3, with the most severe spermatogenic dysfunction, had SPT scores less than 0.25 and sperm densities of less than 1 (0.65 ± 0.31) million/ml. Administration of pulsatile LHRH (4 μg every 120 minutes) for a period of 6 months significantly increased sperm density (with three pregnancies) and serum LH levels and decreased serum FSH levels in men with less severe degrees of spermatogenic damage (i.e., groups 1 and 2). In contrast, men with severe testicular damage (group 3) failed to show significant changes in sperm density or FSH levels.

These results of Aulitsky et al. suggest that some men with idiopathic oligospermia and selective elevations in FSH levels may benefit from pulsatile LHRH treatment. Furthermore, they suggest that baseline assessment of the severity of the spermatogenic disorder (e.g., by SPT scores) may be useful in selecting patients who are more likely to respond to therapy. These findings may also help to explain why we and Bals-Pratsch et al.,[38] who studied men with severe spermatogenic dysfunction, failed to show a stimulatory effect of pulsatile LHRH therapy on sperm production.

None of the studies investigating pulsatile LHRH treatment of men with idiopathic oligoazoospermia included placebo-treated control subjects. Because sperm counts exhibit marked within-individual fluctuations in men with oligospermia, such controls are essential in determining whether pulsatile LHRH therapy indeed has a stimulatory effect on spermatogenesis. Because no other therapeutic options exist, the use of pulsatile LHRH therapy for men with idiopathic oligospermia and selective elevations of FSH levels deserves to be evaluated critically in formal double-blind, placebo-controlled clinical trials. Pulsatile LHRH treatment is the only promising new therapeutic modality on the horizon for this common and, at the present time, untreatable disorder.

In summary, using frequent measurement of blood LH levels to reflect episodic LHRH release, two major disorders of the LHRH pulse generator have been characterized in men: (1) men with hypogonadotropic eunuchoidism, most of whom lack detectable LH pulses and presumably have LHRH deficiency, and (2) men with idiopathic oligoazoospermia with selective elevations in serum FSH levels many of whom demonstrate decreased spontaneous LH pulse frequency, presumably as a result of slowed LHRH pulse generation.

In men with hypogonadotropic eunuchoidism long-term low-dose (physiologic) pulsatile LHRH administration is effective in restoring normal pituitary gonadotropin and testicular steroid and sperm production. In contrast, continuous administration of a physiologic LHRH dosage, although increasing immunoreactive LH levels, does not stimulate bioactive LH levels or FSH levels. Therefore a pulsatile LHRH stimulus pattern is an absolute requirement for normal pituitary gonadotropin secretion in men, and long-term low-dose continuous LHRH treatment is unlikely to stimulate normal testicular function.

In men with idiopathic oligoazoospermia and selective elevation of FSH levels, long-term pulsatile LHRH administration is effective in decreasing serum FSH levels. However, the efficacy of LHRH treatment in stimulating spermatogenesis and improving fertility in these men is unclear. Because this therapeutic modality holds promise as a useful treatment for a common, presently untreatable disorder, it deserves further formal clinical investigation.

REFERENCES

1. Arimura A, Kastin AJ, Gonzalez-Barcena D, Siller J, Weaver RE, Schally AV. Disappearance of LHRH in man as determined by radioimmunoassay. Clin Endocrinol (Oxf) 1974;3:421.

2. Clarke IJ, Cummins JT. The temporal relationship between gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretions in ovariectomized ewes. Endocrinology 1982;111:1737.
3. Levine JE, Pau K-YF, Ramirez VO, Jackson GL. Simultaneous measurement of luteinizing hormone–releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. Endocrinology 1982;111: 1449.
4. Matsumoto AM, Bremner WJ. Modulation of pulsatile gonadotropin secretion by testosterone in man. J Clin Endocrinol Metab 1984;58:609.
5. Crowley WF Jr, Filicori M, Spratt D, Santoro N. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. Recent Prog Horm Res 1985;41:473.
6. Kallmann FJ, Schonfeld WA, Barrera SE. The genetic aspects of primary eunuchoidism. Am J Ment Defic 1944;48:203.
7. Santen RJ, Paulsen CA. Hypogonadotropic eunuchoidism. I. Clinical study of the mode of inheritance. J Clin Endocrinol Metab 1973;36:47.
8. Lieblich JM, Rogol AD, White BJ, Rosen SW. Syndrome of anosmia with hypogonadotropic hypogonadism. Am J Med 1982;73:506.
9. Spratt DI, Carr DB, Merriam GR, Scully RE, Rao RN, Crowley WF. The spectrum of abnormal patterns of gonadotropin-releasing hormone secretion in men with idiopathic hypogonadotropic hypogonadism: clinical and laboratory correlations. J Clin Endocrinol Metab 1987; 64:283.
10. deKretser DM, Berger HG, Fortune D, et al. Hormonal, histological and chromosomal studies in adult males with testicular disorders. J Clin Endocrinol Metab 1972;35: 392.
11. Rosen SW, Weintraub BD. Monotropic increase of serum FSH correlated with low sperm count in young men with idiopathic oligospermia and aspermia. J Clin Endocrinol Metab 1971;32:410.
12. Kulin HE, Reiter EO. Gonadotropin suppression by low-dose estrogen in men: evidence for differential effects upon FSH and LH. J Clin Endocrinol Metab 1972;35:836.
13. Gross KM, Matsumoto AM, Southworth MB, Bremner WJ. Evidence for decreased luteinizing hormone–releasing hormone pulse frequency in men with selective elevations of follicle-stimulating hormone. J Clin Endocrinol Metab 1985;60:197.
14. Wagner TOF, Brabant G, Warsch F, von Zur Mühlen A. Slow pulsing oligospermia. [Abstract]. Acta Endocrinol Suppl (Copenh) 1983;105:152.
15. Matsumoto AM, Bremner WJ. Hypogonadism: androgen therapy. In: Krieger DT, Bardin CW, eds. Current therapy in endocrinology and metabolism 1985-1986. Philadelphia: BC Decker, 1985:145.
16. Paulsen CA, Espeland DH, Michals EL. Effects of hCG, hMG, hLH, and hGH administration on testicular function. In: Rosemberg E, Paulsen CA, eds. The human testis. New York: Plenum Press, 1970:547.
17. Sherins RJ. Evaluation and management of men with hypogonadotropic hypogonadism. In: Garcia CR, Mastroianni L, Amelar RD, Dubin L, eds. Current therapy in infertility. Philadelphia: BC Decker, 1984:147.
18. Ley SB, Leonard JM. Male hypogonadotropic hypogonadism: factors influencing response to human chorionic gonadotropin and human menopausal gonadotropin, including prior exogenous androgens. J Clin Endocrinol 1987;61:746.
19. Finkel DM, Phillips JL, Snyder PJ. Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. N Engl J Med 1985;313:651.
20. Burger HG, deKretser DM, Hudson B, Wilson JD. Effects of preceding androgen therapy on testicular response to human pituitary gonadotropin in hypogonadotropic hypogonadism: a study of three patients. Fertil Steril 1981;35:64.
21. Whitcomb RW, Crowley WF Jr. Diagnosis and treatment of isolated gonadotropin-releasing hormone deficiency in men. J Clin Endocrinol Metab 1990;70:3.
22. Hoffman AR, Crowley WF. Induction of puberty in men by long-term pulsatile administration of low-dose gonadotropin-releasing hormone. N Engl J Med 1982; 307:1237.
23. Belchetz PE, Plant TM, Nakai Y, Keough EJ, Knobil E. Hypophyseal responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science 1978;202:631.
24. McNeil LW, McKenna TJ, Lacroix A, Benveniste R, Rabin D. Seventy-two hour infusions of LHRH in normal men: gonadotropin and testicular steroid responses. J Clin Endocrinol Metab 1979;49:149.
25. Rabin D, McNeil LW. Pituitary and gonadal desensitization after continuous luteinizing hormone–releasing hormone infusion in normal females. J Clin Endocrinol Metab 1980;51:873.
26. Labrie F, Dupont A, Bélanger A, et al. Treatment of prostate cancer with gonadotropin-releasing hormone agonists. Endocrinol Rev 1986;7:67.
27. Matsumoto AM, Gross KM, Bremner WJ. The physiological significance of pulsatile LHRH secretion in man: gonadotropin responses to physiological doses of pulsatile versus continuous LHRH administration. Int J Androl (in press).
28. Gross KM, Matsumoto AM, Berger RE, Bremner WJ. Increased frequency of pulsatile luteinizing hormone–releasing hormone administration selectively decreases follicle-stimulating hormone levels in men with idiopathic azoospermia. Fertil Steril 1986;45:392.
29. Hönigl W, Knuth UA, Nieschlag E. Selective reduction of elevated FSH levels in infertile men by pulsatile LHRH treatment. Clin Endocrinol (Oxf) 1986;24:177.
30. Wildt L, Häusler A, Marshall G, et al. Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. Endocrinology 1981;109:376.
31. Wu FCW, Irby DC, Clarke IJ, Cummins JT, deKretser DM. Effects of gonadotropin-releasing hormone pulse-frequency modulation on luteinizing hormone, follicle-stimulating hormone and testosterone secretion in hypothalamo/pituitary disconnected rams. Biol Reprod 1987;37:501.
32. Adams LA, Clifton DK, Bremner WJ, Steiner RA. Testosterone modulates the differential release of luteinizing hormone and follicle-stimulating hormone that occurs in response to changing gonadotropin-releasing hormone pulse frequency in the male monkey, *Macaca fascicularis*. Biol Reprod 1988;38:156.
33. Gross KM, Matsumoto AM, Bremner WJ. Differential control of luteinizing hormone–releasing hormone pulse frequency in man. J Clin Endocrinol Metab 1987;64:675.
34. Haisenleder DJ, Khoury S, Zmelili SM, et al. The frequency of gonadotropin-releasing hormone secretion regulates expression of α and luteinizing hormone β-subunit messenger ribonucleic acids in male rats. Mol Endocrinol 1987;1:834.
35. Gordon DL, Moore DL, Thorslund T, Paulsen CA. The determination of size and concentration of human sperm with an electronic particle counter. J Lab Clin Med 1965;65:506.
36. Bremner WJ, Matsumoto AM, Sussman AM, Paulsen CA. Follicle-stimulating hormone and human spermatogenesis. J Clin Invest 1981;68:1044.
37. Matsumoto AM, Paulsen CA, Hopper BR, Rebar RW, Bremner WJ. Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors and sperm production despite high estradiol levels. J Clin Endocrinol Metab 1983;56:720.

38. Bals-Pratsch M, Knuth UA, Hönigl W, Klein HM, Bergman M, Nieschlag E. Pulsatile GnRH-therapy in oligozoospermic men does not improve seminal parameters despite decreased FSH levels. Clin Endocrinol (Oxf) 1989;30:549.
39. Wagner TOF, von zur Mühlen A. Slow pulsing oligospermia: treatment by longtime pulsatile LHRH therapy. In: Wagner TOF, Filicori M, eds. Episodic hormone secretion: from basic science to clinical application. Hemelin, West Germany: TM Verlag, 1987:197.
40. Aulitzky W, Frick J, Hadziselimovic F. Pulsatile LHRH therapy in patients with oligozoospermic and disturbed LH pulsatility. Int J Androl 1989;12:265.

Discussion

Audience. In your study of men with low sperm count, did you characterize motility, function, and morphology?

Dr. Matsumoto. The few individuals we have studied had a varying spectrum of motility. Most of them had normal motility. According to an in vitro assay of sperm function, the zona-free hamster ova sperm penetration test, the majority of these men had low-normal to reduced sperm penetration. Many have had abnormalities in morphology as well. None of the individuals had varicoceles and, from what we could derive from their histories, none had exposure to any testicular toxins.

Audience. Were you able to document any morphologic change in sperm during the course of therapy with gonadotropin-releasing hormone?

Dr. Matsumoto. We do not have a sufficient number of individuals who have been treated long term from which to draw any firm conclusions.

Gonadotropin-releasing hormone deficiency in men: Diagnosis and treatment with exogenous gonadotropin-releasing hormone

William F. Crowley, MD, and Randall W. Whitcomb, MD

Boston, Massachusetts

Idiopathic hypogonadotropic hypogonadism in men is the result of absent or abnormal secretion of gonadotropin-releasing hormone, which prevents pubertal development. Isolated gonadotropin-releasing hormone deficiency is clinically and neuroendocrinologically heterogeneous, largely because of variation in the degree of gonadotropin-releasing hormone deficiency. Treatment with pulsatile gonadotropin-releasing hormone results in normal pubertal changes and virilization. Such treatment has been uniformly successful in normalizing release of gonadotropins and secretion of testosterone. Improvement in secondary sex characteristics and induction of spermatogenesis have been achieved in most patients. Eight of nine patients desiring induction of fertility were able to father a child. Use of pulsatile gonadotropin-releasing hormone presents a powerful model in which to examine regulation of gonadotropin secretion and the role of hypothalamic gonadotropin-releasing hormone in control of the male reproductive axis. (Am J Obstet Gynecol 1990;163:1752-8.)

Key words: Gonadotropin-releasing hormone in men, idiopathic hypogonadotropic hypogonadism, apulsatile luteinizing hormone pattern, developmental arrest luteinizing hormone pattern, decreased amplitude luteinizing hormone pattern

Men with idiopathic hypogonadotropic hypogonadism fail to undergo puberty because of a deficiency in the endogenous secretion of the hypothalamic gonadotropin-releasing hormone (GnRH). Episodic discharge of GnRH appears to be the central event in the initiation of puberty. In GnRH deficiency, secondary sex characteristics fail to develop normally, and prepubertal gonadotropin and testosterone levels persist into adult life.[1]

From the Department of Reproductive Endocrinology, Massachusetts General Hospital.
Supported by National Institutes of Health grant ROIHD15788.
Reprint requests: William F. Crowley, MD, Department of Reproductive Endocrinology, Massachusetts General Hospital, Bartlett Hall Extension 5, Boston, MA 02114.
6/0/24548

Clinical patterns of GnRH deficiency

There are several lines of evidence that indicate the existence of a number of different forms of idiopathic hypogonadotropic hypogonadism. These include different modes of inheritance, the inconsistent association of other anomalies, and varying degrees of sexual development and responsiveness to GnRH testing in

men with idiopathic hypogonadotropic hypogonadism. The occasional development of idiopathic hypogonadotropic hypogonadism after puberty suggests that it may also occur as an acquired disorder. There appears to be greater variability therefore in clinical and neuroendocrine presentation than is generally realized.[2, 3] This is largely the result of varying degrees of GnRH deficiency.[3]

The most common clinical presentation is the complete absence of pubertal development with minimal testicular growth.[3] Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and plasma testosterone levels are usually below the normal male range. A more severely affected subgroup of these patients has anosmia, microphallus, and incompletely descended testes with serum gonadotropin levels at or below assay detection limits.[2] This has frequently been called the Kallmann's syndrome, and genetic association with color blindness, anosmia, synkinesis, and mental defect has been described.[4] GnRH secretion may have been deficient in utero in the more severely affected group, to the extent that testosterone production was stimulated by maternal chorionic gonadotropin alone.[2]

An uncommon subset of men with isolated GnRH deficiency has a history of normal puberty and partial sexual development, but it has a subsequent decrease in libido and infertility. Hypogonadotropic hypogonadism usually develops between the ages of 20 and 35 years, with testicular volume near or within the normal adult range and biopsy evidence of normal spermatogenesis. Apparently these men have either a partial or acquired form of idiopathic hypogonadotropic hypogonadism.[2, 3]

The so-called "fertile eunuch" syndrome represents still another subset of patients in which GnRH deficiency is incomplete. There appears to be sufficient secretion of GnRH and resultant gonadotropin output to stimulate spermatogenesis and some testicular growth. There is a marked deficiency of androgen production, however, and a virtual absence of mature Leydig cells. Gonadotropin secretion is inadequate to stimulate Leydig cell production of testosterone required for full virilization.[3] Plasma FSH concentrations are in the normal adult male range, but LH levels are low.

Genetic considerations

Several studies have described kindreds with GnRH deficiency compatible with an autosomal-dominant or an autosomal-recessive mode of inheritance. In our experience with 120 consecutively studied patients, however, the majority of cases were sporadic.[3]

The defect in idiopathic hypogonadotropic hypogonadism appears to occur at a suprapituitary level and

Table I. Pulse characteristics in normal men*

Pulse criteria (mU/ml)	*Frequency (pulses/24 hr)*	*Amplitude (mU/ml second IRP)*
>1	12.6 ± 1.0	9.4 ± 0.9
>2	12.0 ± 1.1	9.7 ± 0.9
>3	10.9 ± 1.0	10.8 ± 0.8

Modified from Crowley WF Jr, et al. Recent Prog Horm Res 1985;41:473.

IRP, International Reference Preparation.

*The characteristics of pulsatile LH secretion in 15 normal men sampled at 10-minute intervals. For identification, a pulse was required to exceed an amplitude of 1, 2, or 3 mU/ml and to consist of at least two consecutive points.

to involve mechanisms that regulate GnRH synthesis and release. A plausible basis for such a defect would be an absent or abnormal GnRH gene.

Yang-Feng et al.[5] recently isolated the gene-encoding biosynthesis of GnRH and localized it to the short arm of chromosome 8. The structure of this gene was studied in 13 patients (11 men and 2 women) with clinically and biochemicaly documented idiopathic hypogonadotropic hypogonadism. No deletions or major rearrangements of the gene could be identified.[6]

Smaller, more subtle defects within the gene remain a possibility. Definite exclusion of point mutations will require cloning and sequencing the gene from a number of affected individuals.

Alternatively, genetic lesions outside the GnRH locus may form the basis for GnRH deficiency in idiopathic hypogonadotropic hypogonadism. Such a lesion might produce a defect in the GnRH-producing neurons or abnormalities in the neural pathways that control GnRH secretion.

Studies in animals[7] have demonstrated that GnRH-producing neurons originate embryologically in the embryonic olfactory placode outside the brain. During fetal development, they migrate to their adult locations in the hypothalamus and the olfactory tract. Defects in the pathways regulating this migration could account for the absence of GnRH secretion, as well as the anosmia observed in many patients with idiopathic hypogonadotropic hypogonadism.[7]

Differential diagnosis

Before making the diagnosis of idiopathic hypogonadotropic hypogonadism, it is important to rule out the more common hypogonadotropic states caused by a mass lesion of the hypothalamic-pituitary area. Magnetic resonance imaging or computed tomography should be an initial step in the workup of all patients with idiopathic hypogonadotropic hypogonadism. The diagnosis of isolated GnRH deficiency can only be made

Table II. Characteristics of subgroups of men with IHH

Group	No. of patients		LH (mU/ml)*	FSH (mU/ml)*	Testosterone (ng/dl)	Testicular volume (ml)
	A	S				
Apulsatile						
−Hx Pub	17	15	1.7 ± 0.3†	2.1 ± 0.2†	50 ± 4†	3.3 ± 0.5†
+Hx Pub	0	10	1.4 ± 0.2†	2.3 ± 0.4†	64 ± 9†	13.3 ± 1.9‡
Developmental arrest	0	3	4.9 ± 0.6§	4.5 ± 2.4§	94 ± 13†	10.5 ± 2.3‡
Decreased amplitude	1	3	1.6 ± 0.5‖	4.0 ± 0.5	50 ± 12	5.6 ± 1.9¶
Normal men	0	20	8.9 ± 1.1	6.7 ± 1.3	635 ± 44	20 ± 1.0

Modified from Spratt DI, Carr DB, Merriam JR, Scully RE, Narasimha Rao P, Crowley WF Jr. The spectrum of abnormal patterns of gonadotropin-releasing hormone secretion in men with idiopathic hypogonadotropic hypogonadism: clinical and laboratory correlations. J Clin Endocrinol Metab 1987;64:283.

Values are the mean ± SE.

A, With anosmia; *S*, without anosmia; *Hx Pub*, history of partial or complete puberty.

*Second International Reference Preparation.

†$p < 0.001$ compared with normal men.

‡$p < 0.01$ compared with men with IHH with no detectable LH pulses or history of spontaneous puberty.

§$p < 0.05$ compared with men with IHH with no detectable LH pulses or history of spontaneous puberty.

‖$p < 0.01$ compared with normal men.

¶$p < 0.05$ compared with normal men.

in the presence of otherwise normal pituitary function and the absence of any anatomic defect.

GnRH secretion in normal men

Driven by the episodic discharge of GnRH, LH is secreted in a pulsatile fashion in men. LH then acts to stimulate production of testosterone by the Leydig cell.

Testosterone acts as the primary hormonal signal regulating secretion of LH in men. It exerts its effect predominantly through negative feedback at the level of the central nervous system and slows the frequency of pulsatile GnRH discharge. This in turn reduces pulsatile LH secretion. Testosterone may also exert a minor negative feedback effect at the anterior pituitary gland, but this is quantitatively less important.

Two features unique to neuroendocrine systems complicate the direct measurement of GnRH secretion. One is the intermittent mode of secretion of releasing factors and their evanescent character. GnRH, in particular, has a half-life of only 2 to 4 minutes. The second obstacle is the nature of the closed blood supply linking the central nervous system to the anterior pituitary gland; only a very small fraction of the GnRH secreted from the hypothalamus ever leaves this limited anatomic space. Thus GnRH levels in the peripheral circulation are very low. As a result of these difficulties, we have used monitoring of LH pulsations as an indirect assessment of GnRH secretion.

GnRH secretion was studied by LH pulsation monitoring in 15 men (aged 18 to 35 years) with a normal history of sexual development, normal testicular size, and normal seminal fluid analysis.[8] Gonadotropin levels were sampled at 10-minute intervals during a 24-hour period. A range of LH pulse frequencies varying from 7 to 17 pulses per 24 hours was observed. Pulse amplitudes and frequencies varied slightly, depending on the pulse criterion employed (Table I). Such data are used for construction of physiologically based programs of exogenous GnRH therapy.[8]

A predominant clustering of the LH pulses during sleeping hours reflects a pattern of GnRH secretion characteristic of puberty that was formerly thought to occur only at that time of life.[9] During repeated studies, however, this pattern appeared to be a reproducible finding in some of our normal subjects.[8]

Physiology of GnRH in idiopathic hypogonadotropic hypogonadism

The genetic, clinical, and biochemical heterogeneity of various subgroups of men with idiopathic hypogonadotropic hypogonadism noted above was suggestive of significant variability in the patterns of GnRH secretion and degree of GnRH deficiency. To test this hypothesis, the patterns of GnRH-induced gonadotropin secretion were characterized in 50 consecutive men with idiopathic hypogonadotropic hypogonadism and compared with those in 20 normal men.[2]

Patterns of pulsatile LH secretion were classified into three broad groups: (1) apulsatile pattern (no detectable gonadotropin pulses), (2) developmental-arrest pattern (LH pulsations occurring predominantly during the night), and (3) decreased-amplitude pattern (LH pulsations of decreased amplitude occurring day and night). Based on histories, physical examinations, and testicular biopsy findings, these groups were further divided into subsets (Table II).

Of the 50 men with idiopathic hypogonadotropic hypogonadism, 42 had an apulsatile pattern with no detectable LH or FSH pulsations. One subset of this group with apulsatile patterns (n = 32; aged 18 to 58 years) recalled no history of puberty, had a mean testicular volume of 3.3 ± 0.5 ml, and had testicular biopsy specimens revealing seminiferous tubules with a prepubertal appearance. Seventeen men in this subset had anosmia, and four had microphallus and incompletely descended testes with volumes of 1 to 2 ml. Serum gonadotropin levels were generally undetectable in these four men.

A second subset of men with apulsatile patterns (n = 10; aged 18 to 39 years) had a history of partial or complete sexual maturation with subsequent isolated idiopathic loss of sexual function. Of the three patients with histories of having normal children, one had previously fathered two children and the other two had isolated losses of potency and libido. In three of the men with histories of only partial puberty, testicular biopsy specimens demonstrated prepubertal histologic findings in the first, arrest of spermatogenesis in the second, and mature spermatogenesis in the third. All 10 of these men had an intact sense of smell, and none had microphallus or undescended testes. Mean pooled serum LH and FSH values were similar to those in the subset with no history of spontaneous puberty (Table II).

Three men (aged 18 to 24 years) had the developmental-arrest pattern of detectable LH pulsations predominantly during the night. One of these patients related the onset of puberty between the ages of 15 and 16 years. This was characterized by spontaneous daytime erections, minimal ejaculations, increased libido, and weekly shaving by age 17 years. There was no progression or decline thereafter. The other two patients recalled no pubertal symptoms. Serum LH and FSH values in these patients were within the lower range of normal and were significantly greater than those in patients with apulsatile patterns (Table II).

The decreased-amplitude pattern was seen in four men (aged 24 to 41 years). These patients had LH pulsations of abnormally low amplitude during both sleeping and waking hours. Their mean pulse amplitude was 0.8 ± 0.1 mU/ml (LER 907), which was significantly lower than the mean value in normal subjects (1.8 ± 0.2 mU/ml; $p < 0.005$). Three brothers who were in this group had an intact sense of smell, testicular volumes of 3 ml or less, and mean pooled serum testosterone levels of 34 ± 5 ng/dl. The fourth man had anosmia with testicular volumes of 12 and 15 ml and a serum testosterone level of 99 ng/dl. Serum LH and FSH levels in this group of patients were significantly below the normal range.

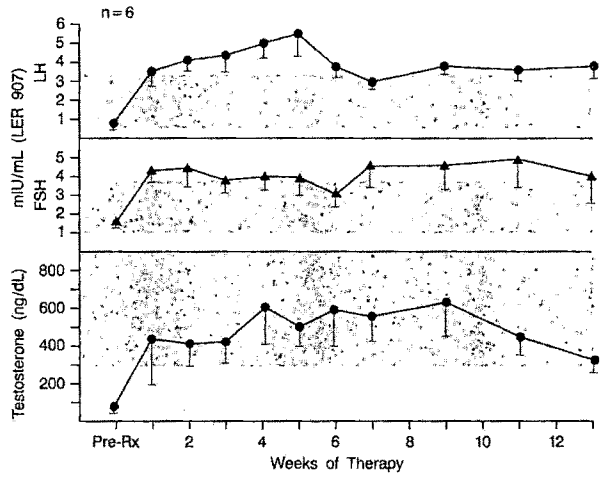


Fig. 1. Mean levels (± SE) of LH, FSH, and testosterone in six men with idiopathic hypogonadotropic hypogonadism. The dosage of GnRH during 3 months of administration was 25 ng/kg given subcutaneously every 2 hours. The *shaded areas* represent the range of normal values in men. The values for weeks 6 and 9 represent those in five of the patients, and the values for week 13 represent those in four patients. (Reprinted by permission of N Engl J Med 1982;307:1237.)

The remaining patient was difficult to classify. The amplitude and frequency of his GnRH-induced gonadotropin pulsations appeared to be normal. His serum LH value, however, was biologically inactive when tested in the dispersed rat Leydig's cell assay. Apparently the defect was the secretion of a biologically inactive LH molecule.[2,8]

We had previously reported two brothers with a relatively slow frequency of LH pulsations. They had severe gynecomastia and serum testosterone levels intermittently below the normal range.[8] The spectrum of GnRH secretory abnormalities seen in idiopathic hypogonadotropic hypogonadism therefore includes a complete absence of LH pulses, as well as disorders of amplitude and frequency, a pubertal arrest pattern, and the presence of bioinactive LH secretion.

Treatment with GnRH

Isolated GnRH deficiency has been treated by intravenous or subcutaneous pulsatile administration of GnRH. Results have been uniformly positive in terms of normalizing gonadotropin and testosterone levels. Most patients have had successful stimulation of puberty and normal virilization.

Intravenous administration produces the most physiologic GnRH pulse contour and ensuing LH response. The circumstances in which long-term intravenous maintenance is feasible, however, are obviously limited. Therefore subcutaneous administration with a programmable peristaltic infusion pump is the preferred route.

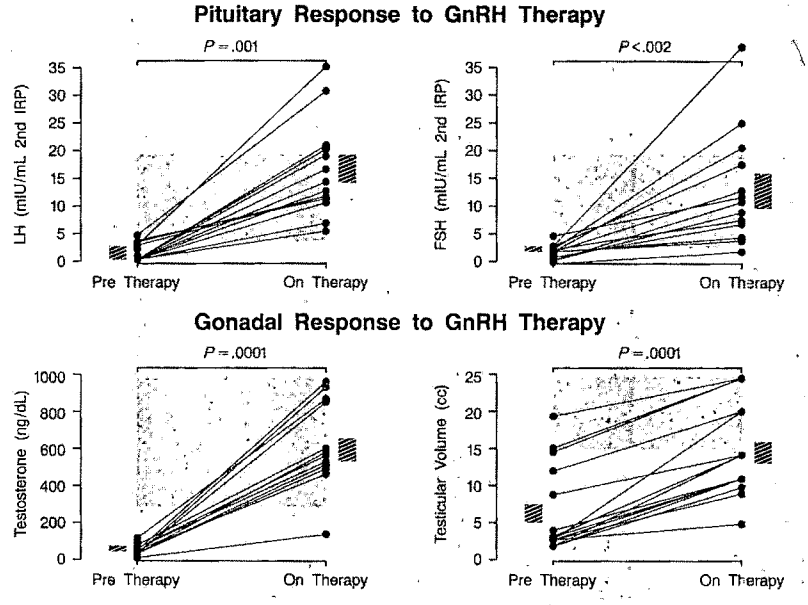


Fig. 2. Pituitary and gonadal responses of subjects with idiopathic hypogonadotropic hypogonadism before and after long-term GnRH administration. In the sole subject whose serum testosterone level did not normalize an antibody to GnRH developed before completion of his sexual maturation. The *shaded areas* indicate normal ranges for men and the *hatched bars* represent mean values for 14 patients. IRP, International Reference Preparation. (Modified from Crowley WF Jr, et al. Recent Prog Horm Res 1985;41:473.)

Administration of GnRH every 2 hours simulates the normal secretion of the endogenous hormone. This frequency of administration has been shown to be effective in GnRH-deficient men. An increase or decrease in frequency of administration results in deviations from the normal levels of both gonadotropin and testosterone. Individual GnRH dosage requirements are quite variable, ranging from 25 to 600 ng/kg in each bolus. Required dosage correlates positively with body weight and negatively with initial testicular volume.

The response of patients' serum testosterone and gonadotropin levels to GnRH administration and the rate of testicular growth should be monitored closely at 2- to 4-week intervals during long-term therapy. Eventually, regular seminal analyses should be performed. Most patients will require treatment for a minimum of 2 years to maximize testicular growth and achieve spermatogenesis.

A 100% success rate in stimulating pituitary gonadotropin output and testicular steroid secretion was observed in a series of 51 consecutive men with GnRH deficiency from the Massachusetts General Hospital.[3] In a pilot study with six patients with idiopathic hypogonadotropic hypogonadism,[1] mean serum LH values peaked during the fifth week of treatment at 4.80 ± 0.89 mU/ml and declined into the high-normal range by the seventh week. The decrease was caused by negative feedback inhibition by the sex steroids. Mean FSH levels increased to 4.77 ± 0.81 mU/ml by week 2 and remained elevated during most of the first 3 months. By the end of the first week, mean serum testosterone levels rose to the adult range and remained elevated for the first 3 months (Fig. 1).

The final pituitary and gonadal responses achieved in a group of patients with idiopathic hypogonadotropic hypogonadism receiving long-term replacement therapy with GnRH in doses ranging from 25 to 200 ng/kg are shown in Fig. 2. Only one patient failed to achieve a normal pituitary and gonadal response. An antibody to GnRH developed in this man before he could attain full sexual maturity. Changes in secondary sex characteristics induced by this regimen are shown in Table III.

Fig. 3 shows the testicular growth response in 17 patients recently treated for a minimum of 24 months with a pulsatile GnRH regimen that was adjusted to normalize both gonadotropin and testosterone secretion. Patients with larger initial gonadal size generally have the most rapid testicular growth. Individual patients with smaller initial gonadal size, however, can also achieve normal testicular volume.[3]

Spermatogenesis was induced successfully in 34 of 51 GnRH-deficient men who submitted samples for analysis during long-term GnRH therapy. Of the remaining 17 patients, two had severe cryptorchidism and failed to achieve significant testicular growth with

Table III. Symptomatic changes in 14 men with IHH during prolonged GnRH administration

Symptom	*n*	%
↑ Libido and potency	14/14	100
↑ Spontaneous erections	14/14	100
↑ Testicular size	13/14	93
Appearance of an ejaculate	11/14	79
↑ Facial hair	9/14	64
↓ Voice	9/14	64
Appearance of mature sperm in ejaculate	7/11	64

Modified from Crowley WF Jr, et al. Recent Prog Horm Res 1985;41:473.

GnRH, three discontinued treatment before significant growth of the testes, three had azoospermia after 8 to 18 months of therapy, three failed to submit specimens for analysis, and six had been receiving therapy less than 6 months.

The time required for appearance of sperm in the ejaculate ranged from 18 to 139 weeks after initiation of therapy and was mainly a function of the size of the pretherapy gonad. Testicular size presumably reflected the degree of seminiferous tubular maturation. Other factors correlating positively with induction of spermatogenesis included duration of therapy, adequacy of dosage, patient compliance, and the absence of cryptorchidism.

Variability of sperm counts as a function of testicular size is illustrated in Fig. 4. Note that it is uncommon to see spermatogenesis with a testicular volume of less than 8 ml. High counts may be seen in patients with smaller testes, however, and low counts may be seen in those with larger testes. This emphasizes the need for close monitoring and careful individualization of therapy.[3]

It is important to recognize that patients treated with GnRH have excellent fertility potential, in spite of subnormal sperm counts. Nine of our GnRH-deficient patients who were seeking fertility fathered eight children. Sperm counts immediately preceding the time of conception ranged from 1 to 68 million/ml, and 75% of conceptions occurred at less than 15 million/ml. The one man who desired fertility and failed to impregnate his wife had a past history of cryptorchidism.

In summary a broad spectrum of abnormal GnRH secretory patterns that underlie idiopathic hypogonadotropic hypogonadism in men has been described. These patterns range from total absence of GnRH secretion through arrested pubertal development to idiopathic loss of GnRH secretion after puberty has been completed. The various subsets of idiopathic hypogonadotropic hypogonadism may be ideal for studying the role of the hypothalamic-pituitary-gonadal axis in control of reproduction.

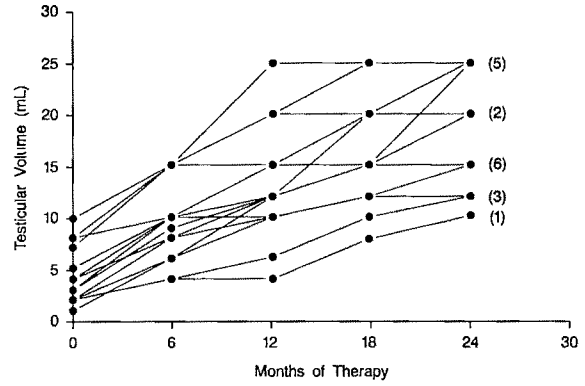


Fig. 3. Testicular growth response of 17 patients with idiopathic hypogonadotropic hypogonadism treated with pulsatile GnRH. The numbers in parentheses represent the number of individuals with that testicular volume after 24 months of pulsatile GnRH therapy. (Modified from Whitcomb RW, Crowley WF Jr. Clinical review 4: diagnosis and treatment of isolated gonadotropin-releasing hormone deficiency in men. J Clin Endocrinol Metab 1990;70:3.)

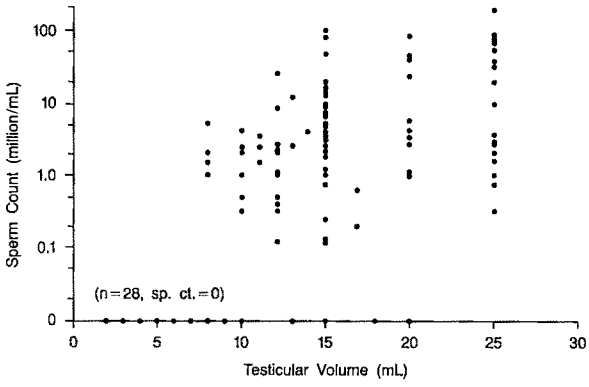


Fig. 4. Results of 128 seminal analyses in patients with idiopathic hypogonadotropic hypogonadism before and during GnRH therapy as a function of testicular volume. Of 28 samples without sperm, 20 were from patients with testicular volumes less than 9 ml. Modified from Whitcomb RW, Crowley WF Jr. Clinical review 4: diagnosis and treatment of isolated gonadotropin-releasing hormone deficiency in men. J Clin Endocrinol Metab 1990;70:3.

Patients with incomplete development of the GnRH secretory pattern may be important models for studying hypothalamic and suprahypothalamic factors influencing GnRH secretion. On the other hand, patients with no evidence of GnRH secretion provide a unique human model for studying the physiology of GnRH stimulation of the anterior pituitary gland through therapeutic administration of exogenous hormone. The information derived can make an important contribution toward greater understanding of the pathophysiology of male reproductive disorders. It may eventually help us design valuable new therapeutic and contraceptive strategies.

Clinically it has been demonstrated that one can achieve full sexual maturation in men with idiopathic hypogonadotropic hypogonadism whose pituitary-gonadal axis can be normalized by long-term administration of exogenous GnRH. Establishment of normal levels of gonadotropin and testosterone secretion is reflected in the development of masculine secondary sex characteristics, increased testicular volume and sperm count, and the induction of fertility in most patients who desire it. The potential personal and social benefits for the patient with idiopathic hypogonadotropic hypogonadism speak for themselves.

We are indebted to Denise Heavern, RN, for her dedicated care of our patients, the patients who volunteered for the studies, the General Clinical Research Center staff, and the technicians of the Reproductive Endocrine Unit.

REFERENCES

1. Hoffman AR, Crowley WF Jr. Induction of puberty in men by long-term pulsatile administration of low-dose gonadotropin-releasing hormone. N Engl J Med 1982; 307:1237.
2. Spratt DI, Carr DB, Merriam GR, Scully RE, Narasimha Rao P, Crowley WF Jr. The spectrum of abnormal patterns of gonadotropin-releasing hormone secretion in men with idiopathic hypogonadotropic hypogonadism: clinical and laboratory correlations. J Clin Endocrinol Metab 1987; 64:283.
3. Whitcomb RW, Crowley WF Jr. Clinical review 4: diagnosis and treatment of isolated gonadotropin-releasing hormone deficiency in men. J Clin Endocrinol Metab 1990;70:3.
4. Kallmann FJ, Schoenfeld WA, Barrera SF. The genetic aspects of primary eunuchoidism. Am J Ment Defic 1944; 48:203.
5. Yang-Feng TL, Seeburg PH, Francke U. Human luteinizing hormone-releasing hormone gene (LHRH) is located on the short arm of chromosome 8 (region 8p11.2→p21). Somat Cell Mol Genet 1986;12:95.
6. Weiss J, Crowley WF Jr, Jameson JL. Normal structure of the gonadotropin-releasing hormone (GnRH) gene in patients with GnRH deficiency and idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 1989; 69:299.
7. Schwanzel-Fukuda M, Pfaff DW. Origin of luteinizing hormone-releasing hormone neurons. Nature 1989;338: 161.
8. Crowley WF Jr, Filicori M, Spratt DI, Santoro NF. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. Recent Prog Horm Res 1985;41:473.
9. Boyar RM, Rosenfeld RS, Kapen S, et al. Human puberty: simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. J Clin Invest 1974;54: 609.

Discussion

Audience. Do you have any explanation of why patients who have hypothalamic hypogonadism can impregnate their wives with very low sperm counts?

Dr. Crowley. I use sperm counts like school. Basically, all we have been doing for years is taking attendance, and we have not had any tests to show what they can do. Now that we have machines that can measure objective parameters, such as lateral head movement, we are going to see that there is quite a heterogeneity in those in attendance.

Audience. Have you used any of the antagonists with subsequent treatment with GnRH?

Dr. Crowley. When you give a GnRH antagonist to a human, you can suppress LH levels. At a maximal dose, where the dose-response curve of inhibition plateaus, LH is suppressed about 70% or 80%. FSH is suppressed only about 30% to 40%, and free α-subunit is suppressed about 30%. When you then give a dose of GnRH, you see a reversal of that hierarchy; you get much more LH than FSH. The doses we have used have been pharmacologic, but on the basis of the response to these doses we would assume that you would get back LH secretion, which is just what you do not want.

On the other hand, the fact that FSH is not suppressed as extensively as LH presents a potential opportunity to use another releasing factor, if one could be identified, for FSH. In other words, give the GnRH antagonist, eliminate the LH and consequent hyperandrogenemia, and then give this FSH-releasing factor, if and when such a factor is discovered.

Efficacy and safety of intravenous pulsatile gonadotropin-releasing hormone: Lutrepulse for Injection

Nanette Santoro, MD
Newark, New Jersey

A multicenter trial was undertaken to assess the ability of pulsatile gonadotropin-releasing hormone to restore physiologic ovulatory function in women with diminished or absent pulsatile gonadotropin-releasing hormone secretion. In 109 women with primary and secondary hypothalamic amenorrhea, pulsatile gonadotropin-releasing hormone (Lutrepulse [gonadorelin acetate] for Injection) was extremely effective, resulting in ovulation in 91% and 96% of patients, respectively. Overall complications of intravenous line placement ranged from 0% to 11% (mean = 7%). A 12% incidence of multiple pregnancy was noted, and a single case of ovarian hyperstimulation was reported in a woman who also received clomiphene citrate. Pulsatile gonadotropin-releasing hormone treatment appears to be of at least comparable efficacy to human menopausal gonadotropins, with the added benefit of reduced overall risks for the patient with hypothalamic amenorrhea. (Am J Obstet Gynecol 1990;163:1759-64.)

Key words: Pulsatile gonadotropin-releasing hormone, primary hypothalamic amenorrhea, secondary hypothalamic amenorrhea

Gonadotropin-releasing hormone (GnRH) is the central hypothalamic signal governing episodic secretion of pituitary gonadotropins (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]). Its obligatory, permissive role in allowing the ovarian processes of folliculogenesis, ovulation, and corpus luteum function to occur was first elucidated in the subhuman primate in the early 1970s.[1] Concurrent clinical studies in women were initiated at the time that the absolute requirement for pulsatile delivery of GnRH was first being discovered.[2] Initial research in women focused on the use of long-acting analogs of GnRH that were thought to be superpotent effectors of ovulation. Unfortunately, the frequent occurrence of down-regulation of pituitary GnRH receptors often interfered with the gonadotropic effects of large doses of GnRH agonist.[3, 4]

The challenge of designing GnRH therapy for women with reproductive disorders has centered around the definition of optimal dosage, frequency, and route of administration for pulsatile administration of this hormone. Because many interspecies differences exist regarding sensitivity to pulsatile GnRH, there was little to guide initial research efforts in determining the appropriate dosage of this peptide, and the precise dosage requirements remain to be established. A useful frequency of GnRH administration in women was initially derived from the successful work in rhesus monkeys.[1] In these studies an hourly frequency of pulsatile GnRH was uniformly successful. However, close scrutiny of the human menstrual cycle[5-7] has allowed for an adjustment to a more physiologic frequency or indeed a panel of frequencies that has now been shown to be effective in adult women. Finally, the optimal route of administration of pulsatile GnRH appears to be intravenous. After an intravenous bolus, a physiologic LH pulse contour is achieved, along with superior rates of ovulation, compared with subcutaneous administration.[8-11] It has become clear, however, that in some cases the intravenous route may pose hazards to a patient,[12] and in selected cases subcutaneous use as a less effective alternative may be considered.

The clinical use of intravenous pulsatile GnRH, which led to its approval by the Food and Drug Administration in the United States, was based on patients in whom this treatment might be expected to be most effective: women with primary hypothalamic amenorrhea, an experiment of nature of near-absolute GnRH deficiency. A second group of women with secondary hypothalamic amenorrhea have also proven to ovulate and become pregnant with great success with this form of treatment, and their data have also been included in this report. Three clinical centers in the United States, along with one large clinical center in Germany, formed most of the contributing data that will be presented in the subsequent tables.[13-16] These centers are broken down and the data are described in

From the Department of Obstetrics and Gynecology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School. Reprint requests: Nanette Santoro, MD, Department of Obstetrics and Gynecology, UMDNJ-New Jersey Medical School, Room MSB E-516, 185 S. Orange Ave., Newark, NJ 07103-2757.
6/0/24549

Table I. Lutrepulse for Injection: dosage ranges for primary hypothalamic amenorrhea

	Center			
	1	*2*	*3*	*4*
Dose range (μg/pulse)	1-8	1-10	2-20	3-20
Mean (μg/pulse)	5.4	5.6	7.9	15.2
*n**	20	21	8	83

*Number of ovulatory regimens.

Table II. Lutrepulse for Injection: efficacy of intravenous therapy

	Center				
	1	*2*	*3*	*4*	*Total*
Primary hypothalamic amenorrhea					
n	11	6	3	14	34
Ovulatory (%)	91	83	100	93	91
Ovulatory regimens (%)					72
Pregnancy (%)					55
Secondary hypothalamic amenorrhea					
n	10	9	9	47	75
Ovulatory (%)	100	89	100	96	96
Ovulatory regimens (%)					82
Pregnancy (%)					62

detail to point up their overall consistency, as well as the contribution of certain idiosyncrasies from center to center, in the overall interpretation.

Material and methods

Patient population. All women recruited from the various centers for study carried the diagnosis of hypothalamic amenorrhea. In most cases this diagnosis referred to amenorrhea of at least 6 months' duration in an individual of normal body weight. Subjects were also required to undergo a clinical examination, with findings consistent with hypothalamic amenorrhea (i.e., no evidence of hirsutism, ovarian enlargement, androgen excess, or hyperprolactinemia). In addition, a normal clinical or biochemical thyroid evaluation and documented absence of hyperprolactinemia were required. Informed consent was obtained from each subject before participation and approval by the respective internal review boards of the institutions involved was obtained. In patients recruited from some of the centers, studies to document tubal patency, semen evaluation of the male partner, and postcoital examinations were performed routinely. However, in other institutions, clinical judgment was exercised as to whether any or all of these tests were carried out.

Protocol. GnRH was administered through an intravenous pump (Zyklomat; Ferring Laboratories, Suffern, N.Y.). Details of this technique have been reported previously.[17] Briefly, all patients received intravenous GnRH (Lutrepulse [gonadorelin acetate] for Injection, Ortho Pharmaceutical Corp., Raritan, N.J.) at the dose ranges outlined in Table I. In general, ovulatory and anovulatory regimens of GnRH lasted for 1 month.

The frequency of the pulsatile GnRH infusion was uniformly 90 minutes in centers 2, 3, and 4. In center 1, a frequency of 90 or 60 minutes was used in the follicular phase, and progressive slowing of the peristaltic pump throughout the luteal phase was employed to mimic the luteal-phase slowing of normal gonadotropin pulsations.[7, 17] These alterations in frequency do not appear to have had an impact on overall pregnancy rates, although the numbers are small (Table II).

Monitoring of subjects. Subjects were monitored with daily blood sampling for LH, FSH, estradiol, and progesterone levels in some centers (1, 2, and 3). Blood samples were obtained 45 minutes after a GnRH dose. Intravenous lines were inspected visually (daily in most cases), and the course of follicular maturation was followed by a combination of (1) serial ultrasonography, (2) examinations of cervical mucus, and (3) charting of basal body temperature.

Laboratory methods. Serum samples from each subject were processed for gonadotropin and sex steroid determination, as described previously for center 1.[17] In Fig. 1 values are expressed as equivalents of the second international reference preparation of human menopausal gonadotropin (milli international units per milliliter). The interassay and intraassay coefficients of variation for FSH were 12.3% and 7.8%; corresponding values for LH were 12.5% and 5.8%.

Results

Practical clinical use of GnRH: efficacy. *Patient selection and route of administration.* The four centers discussed herein used relatively stringent criteria for patient selection. However, each center may have had slight differences in patient selection. Most of the centers employed as study subjects only those women with primary or secondary hypothalamic amenorrhea who suffered solely from an ovulatory defect and had no other contributing infertility factors. This was not true of every center; therefore, pregnancy rates would be expected to be lower in couples with multiple contributory infertility factors. Table II points out the overall clinical results from each of the four centers. Note the consistency of ovulation among all of these patients. With intravenous use, ovulation rates were routinely in excess of 90%. The route of administration of pulsatile GnRH in all of these cases was intravenous, and ancillary data from the three groups reporting on a smaller number of patients receiving subcutaneous GnRH revealed lower efficacy, as has been reported by other investigators.[18-20]

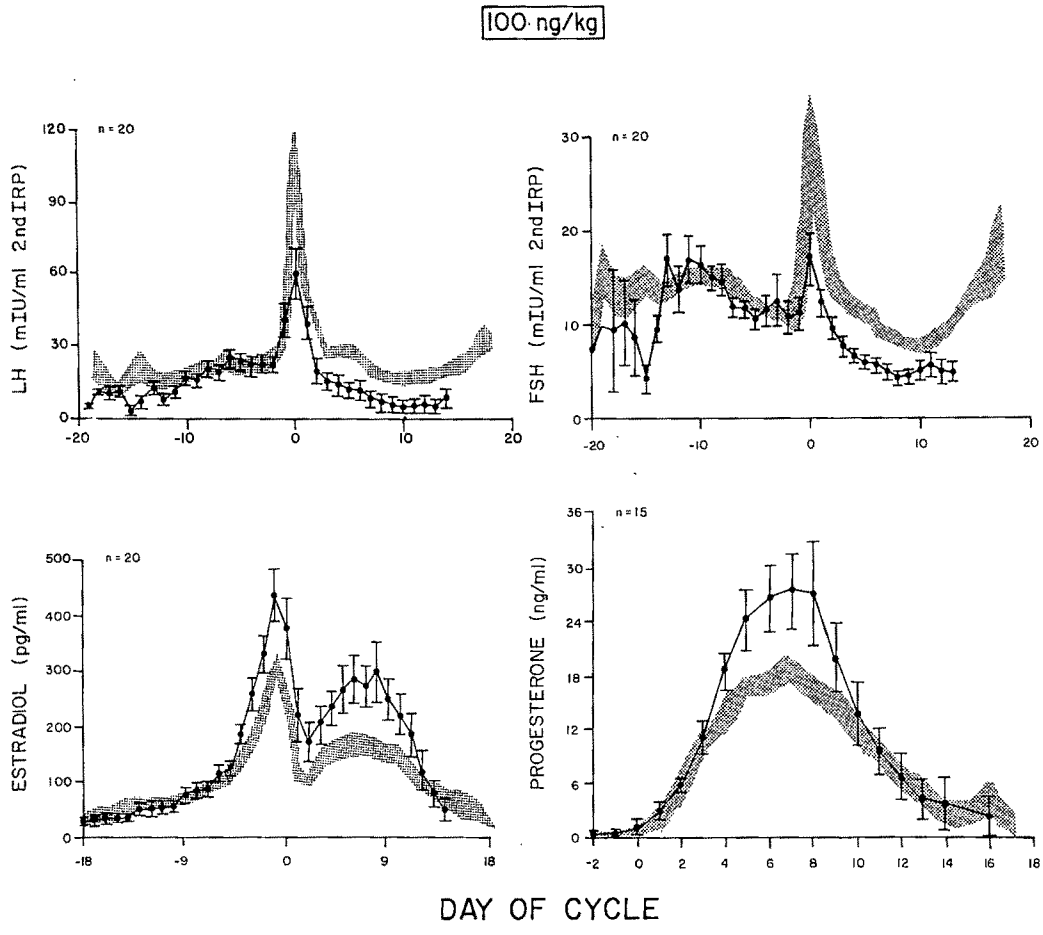


Fig. 1. Ovarian and pituitary responses to 100 ng/kg (approximately 5 μg/bolus) pulsatile GnRH in women with hypothalamic amenorrhea. Normal hormonal data are shown by the shaded background. Data are expressed as mean ± SEM and standardized to the day of the midcycle surge (day 0). The number of patients contributing data to each graph is shown in the upper left. Note that despite normal-appearing gonadotropin levels, supraphysiologic estradiol and progesterone levels were achieved. (From Santoro N, Wierman ME, Filicori M, et al. Intravenous administration of pulsatile gonadotropin-releasing hormone in hypothalamic amenorrhea: effects of dosage. J Clin Endocrinol Metab 1986;62:109.)

Frequency of pulsatile GnRH. The frequency of pulsatile GnRH used was almost uniformly 90 minutes. Group 1 used a varying frequency throughout the cycle, but the choice of a varying frequency did not seem to affect rate of ovulation (Table II). In particular, the follicular-phase frequencies employed by group 1 were 90 or 60 minutes, probably both well within the range of acceptable frequencies tolerated by the female hypothalamic-pituitary-ovarian axis.

Dose of pulsatile GnRH (Table I). The dosage of pulsatile GnRH that has proved successful varies from 1 to 20 μg/bolus. Because the exact quantity of GnRH secreted by the human hypothalamus has been difficult to determine, experimental results directed at observing sex steroid and gonadotropin secretion during pulsatile GnRH-induced cycles are most useful for deriving a physiologic dose. In 18 women studied for 30 cycles at an approximate dose of either 1 or 5 μg/bolus of GnRH, it was found that a 1 μg dose was subphysioilogic and and a 5 μg dose appeared to be slightly supraphysiologic (Fig. 1).[17] One of the other groups contributing to these data noted that at higher doses (between 10 and 20 μg/bolus) of pulsatile GnRH, pituitary and gonadal responses were exaggerated, often suggestive of multiple folliculogenesis, and engendered a higher multiple pregnancy rate of 4 per 30 conceptions.[21] Furthermore, individual sensitivity to a given

Table III. Lutrepulse for Injection: luteal-phase support

	hCG alone	*Lutrepulse alone*	*hCG + Lutrepulse*
Delivered *(n)*	43	19	19
Aborted *(n)*	20	7	6
Total *n*	63	26	25
% success	68	73	76

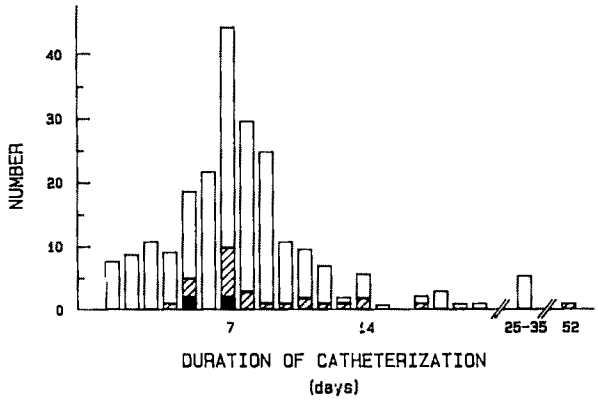


Fig. 2. Catheter tip and blood culture results over 1958 catheter days in relation to duration of catheterization. *Open bars*, Total number of catheter tips assessed; *striped bars*, Number with positive catheter tip cultures; *solid bars*, number of positive blood cultures obtained at the time of catheter removal. (From Hopkins CC, et al. Obstet Gynecol 1989;74:267.)

Table IV. Lutrepulse for Injection: incidence of intravenous complications

	Site irritation		*Superficial thrombophlebitis*	
Center	*n*	%	*n*	%
1	5/132	4	4/132	3
2	0/153	0	1/153	0.7
3	3/35	9	1/35	3
4	49/463	11	15/463	3
Mean		7		3

dose of pulsatile GnRH may require individualization of doses once a supposedly physiologic dosage range is derived. Thus the clinician intending to use this form of treatment will probably need to become conversant with the full dose range that will be made available.

Luteal support. Support of the corpus luteum once ovulation has been induced by pulsatile GnRH appears to be an optional matter. The varying groups used a variety of strategies to support the corpus luteum, all of which appeared equivalent, as can be seen in Table III. Thus one has the option of (1) continuing the pulsatile GnRH infusion throughout the luteal phase; (2) removing the pump as soon as the luteal phase is established (by a shift in basal body temperature, ovulation-predictor kit testing, or hormonal testing) and supplementing with human chorionic gonadotropin (hCG); or (3) employing both modalities until a missed menses, positive pregnancy test result, or menstrual period occurs.

Clinical use of pulsatile GnRH: safety of intravenous pulsatile GnRH. *Safety of the intravenous catheter.* Recent data[12] suggest that the intravenous route of GnRH administration is quite safe for prolonged periods of time. Fig. 2 shows the low incidence of positive catheter tip (11%) and blood cultures (2%) in a group of 38 women receiving long-term pulsatile GnRH with 230 catheter insertions. No clinical evidence of infection attributable to the presence of the intravenous catheter occurred in this series. A small number of cases of superficial thrombophlebitis occurred in patients from all of the four centers analyzed (Table IV). Note the overall consistency of these numbers and the absence of sepsis or major morbidity caused by the presence of the intravenous catheter. Of theoretic concern is the patient with mitral valve prolapse or an abnormal cardiac valve who might be prone to the development of bacterial endocarditis should any bacteremia occur. This theoretic concern can be avoided by careful selection of patients and evaluation of the cardiac examination and echocardiographic findings in patients with heart murmurs undergoing therapy. A woman with mitral valve prolapse with no ballooning or redundancy of the valve, who is considered at lower risk for bacteremia, may well tolerate short-term intravenous use of GnRH with hasty removal of the catheter on ovulation and luteal support with hCG without mishap. However, patients with such defects might well profit from choosing the subcutaneous route, although it will be expected to be less effective.

Table V. Lutrepulse for Injection: complications

Multiple pregnancies
- 11/89 Deliveries (12%)
- 10 Sets of twins
- 1 Set of triplets
- One from center 1 (6.9 μg/pulse: all others reported from center 4 (2.5-20 μg/pulse)

Ovarian hyperstimulation
- 1/268 Patients = 0.4%; 1/932 cycles = 0.1% (subject also received clomiphene citrate)

No patient with primary hypothalamic amenorrhea had a multiple pregnancy or ovarian hyperstimulation

Ovarian hyperstimulation. Only one patient had any clinically significant ovarian hyperstimulation, for an overall incidence of 0.4%. This was of a moderate degree and, of note, this patient had already received clomiphene citrate in conjunction with the pulsatile GnRH. Thus it is expected to be extremely unusual to observe ovarian hyperstimulation of the magnitude that was routinely noted in up to 20% of women who received exogenous human menopausal gonadotropin.[22]

Multiple pregnancy. In Table V, adverse effects of pulsatile GnRH are delineated, including the occurrence of 11 multiple pregnancies. Of these 11 pregnancies, 10 were twin gestations and 1 was a triplet gestation. Table VI points out the data from two centers con-

Table VI. Lutrepulse for Injection: follicle numbers seen on ultrasonography

	Center 1 (n)		*Center 2 (n)*		
No. of follicles	*25 ng/kg (1 μg/bolus)*	*100 ng/kg (5 μg/bolus)*	*Primary hypothalamic amenorrhea*	*Secondary hypothalamic amenorrhea*	*Other*
1	8	15	13	13	44
2	0	4	3	3	4
3	0	1	1	0	2
4	0	0	2	0	0

cerning the number of follicles visualized on routine ultrasound examination. Note that regardless of the primary or secondary nature of the hypothalamic amenorrhea, one to four mature follicles were noted on pelvic ultrasound examination. This supraphysiologic response to pulsatile GnRH may well be an effect of individual differences in requirements for a truly physiologic dose or may reflect a general trend to deliver a supraphysiologic dose to most patients. Fig. 1 shows the pituitary and ovarian responses to an approximately 5 μg/bolus dose of pulsatile GnRH, which underscores the slightly supraphysiologic nature of even the "low-dose" 5 to 6 μg regimen.[17]

Mechanical failure. Of the four centers reporting data, only three recorded the occurrence of mechanical or equipment failure that led to difficulties with treatment (Table VII). Note that in center 4 most of the problems associated with pulsatile GnRH therapy were related to pump malfunctions and the occurrence of superficial thrombophlebitis. They reported the lowest incidence of malfunctions. In center 1 most of the technical problems reported were related to reservoir and tubing defects, not the pump itself. The incidence of superficial thrombophlebitis varies somewhat among the three groups and is perhaps related to the lack of firm criteria for superficial thrombophlebitis and the difficulty distinguishing thrombophlebitis from a simple intravenous infiltration. It is safe to say that at this point the manufacturer has paid a great deal of attention to the tubing and reservoir apparatus and this now rarely malfunctions. Thus the clinician expecting to use this equipment might expect the biggest difficulty to occur with the occasional failure of the peristaltic pump to perform properly.

Table VII. Lutrepulse for Injection: device problems

Center	*% Regimens*	*Primary problem*
1	25	Reservior leakage
2	—	—
3	12	Pump-related
4	3.4	Pump-related
Mean	7.6	

Comment

Pulsatile GnRH is a therapeutic modality whose time has come. It is highly effective in inducing ovulation in women with primary and secondary hypothalamic amenorrhea and routinely induces ovulation in more than 90% of patients. In patients without ancillary infertility factors, pregnancies may be anticipated to occur within several months, and most pregnancies occur within 3 months of therapy. Supplying the hypothalamic hormone to women who appear to be selectively deficient provides a physiologic avenue for therapy that is intuitively attractive to the patient and provides excellent results in terms of single folliculogenesis, single pregnancy, and predictable outcome. In addition to its high rate of efficacy, this form of treatment appears to be very safe even for prolonged intravenous use. The pitfalls of human menopausal gonadotropin therapy, including ovarian hyperstimulation syndrome and multiple pregnancies, appear to be reduced dramatically when pulsatile GnRH is used. Further reduction in dosage and attention to the individual dosage requirements of patients may allow for further reduction of the incidence of ovarian hyperstimulation and multiple pregnancy. The clinician would do well to become acquainted with this new treatment modality, because its indications may well expand into other ovulatory reproductive disorders (e.g., polycystic ovarian syndrome) as an effective and safe alternative to the armamentarium of medications that currently exist.

REFERENCES

1. Knobil E. The neuroendocrine control of the menstrual cycle. Recent Prog Horm Res 1980;36:53.
2. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophyseal responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasisng hormone. Science 1978;202:631.
3. Hammond CB, Wiebe RH, Haney AF, Yancy SG. Ovulation induction with luteinizing hormone-releasing hormone in amenorrheic, infertile women. AM J OBSTET GYNECOL 1979;135:924.
4. Zarate A, Canales ES, Schally AV, Ayala-Valdes L, Kastin AJ. Successful induction of ovulation with synthetic lu-

teinizing hormone-releasing hormone in anovulatory infertility. Fertil Steril 1972;23:672.
5. Yen SSC. Pulsatile gonadorelin acetate in the treatment of hypothalamic amenorrhea. NDA application. Ortho Pharmaceutical Corporation, data on file.
6. Reame N, Saunder SE, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion during the human menstrual cycle: evidence for altered frequency of gonadotropin-releasing hormone secretion. J Clin Endocrinol Metab 1984;59:328.
7. Filicori M, Santoro N, Merriam GR, Crowley WJ Jr. Characterization of the physiologic pattern of episodic gonadotropin secretion throughout the human menstrual cycle. J Clin Endocrinol Metab 1986;62:1136.
8. Handelsman DJ, Swerdloff RS. Pharmacokinetics of gonadotropin-releasing hormone and its analogs. Endocrinol Rev 1986;7:95.
9. Spratt DI, Crowley WJ Jr, Butler JF, Hoffman AR, Conn PM, Badger TM. Pituitary luteinizing hormone responses to intravenous and subcutaneous administration of gonadotropin-releasing hormone in men. J Clin Endocrinol Metab 1985;61:890.
10. Reid RL, Leopold GR, Yen SSC. Induction of ovulation and pregnancy with pulsatile luteinizing hormone-releasing factor: dosage and mode of delivery. Fertil Steril 1981;36:553.
11. Jansen RPS, Handelsman DJ, Boylan LM, Conway A, Shearman RP, Fraser IS. Pulsatile intravenous gonadotropin-releasing hormone ovulation induction in infertile women. I. Safety and effectiveness with outpatient therapy. Fertil Steril 1987;48:33.
12. Hopkins CC, Hall JE, Santoro NF, Martin KA, Filicori M, Crowley WF. Closed intravenous administration of gonadotropin-releasing hormone: Safety of extended peripheral intravenous catheterization. Obstet Gynecol 1989;74:267.
13. Crowley WJ, Filicori M, Santoro N, Wierman M. Pulsatile gonadorelin acetate in the treatment of hypothalamic amenorrhea. NDA application. Ortho Pharmaceutical Corporation, data on file.
14. Leyendecker G, Berg D. Pulsatile gonadorelin acetate in the treatment of hypothalamic amenorrhea. NDA application. Ortho Pharmaceutical Corporation, data on file.
15. Collins RL, Spratt D, Jaffe RB, et al. Pulsatile gonadorelin acetate in the treatment of hypothalamic amenorrhea. NDA Application. Ortho Pharmaceutical Corporation, data on file.
16. Yen SSC. Pulsatile gonadorelin acetate in the treatment of hypothalamic amenorrhea. NDA Application. Ortho Pharmaceutical Corporation, data on file.
17. Santoro N, Wierman ME, Filicori M, et al. Intravenous administration of pulsatile gonadotropin-releasing hormone in hypothalamic amenorrhea: effects of dosage. J Clin Endocrinol Metab 1986;62:109.
18. Hurley DM, Brian R, Outch K, et al. Induction of ovulation and fertility in amendorrheic women by pulsatile low-dose gonadotropin-releasing hormone. N Engl J Med 1984;310:1069.
19. Seibel MM, Kamrava M, McArdle C, Taymor ML. Ovulation induction and conception using subcutaneous pulsatile luteinizing hormone-releasing hormone. Obstet Gynecol 1983;61:292.
20. Skarin G, Nillius SJ, Wide L. Pulsatile low-dose luteinizing hormone-releasing hormone treatment for induction of follicular maturation and ovulation in women with amenorrhea. Acta Endocrinol 1982;101:78.
21. Leyendecker G, Wildt L. Pulsatile administration of GnRH in hypothalamic amenorrhea. Ups J Med Sci 1984;89:19.
22. Gemzell CA, Diczfalusy E, Tillinger G. Clinical effect of human pituitary follicle-stimulating hormone (FSH). J Clin Endocrinol Metab 1958;18:1333.

Discussion

Audience. What is the minimum amount of hCG required to support the luteal phase?

Dr. Santoro. My bias is to keep the pump in throughout the luteal phase because most of the patients really want to find out as early as possible if they are pregnant. By continuing with the pump, you can get an early measurement of hCG. I do not have a great deal of experience with hCG support of the luteal phase, but I have used doses on the order of 500 IU every couple of days in other patients undergoing ovulation induction.

Audience. Would you comment on antibody formation against GnRH?

Dr. Santoro. I think there have been two reported cases of antibody formation in men, and I know of one case that was reported as a possible reaction in a woman. The duration of treatment in men is so much greater that I think they are more likely to have a problem. In addition, the peptide is so small that it seems to be only very weakly antigenic.

Audience. In the woman with hypothalamic amenorrhea who has an infantile uterus, is there any reason to consider pretreatment with estrogen for several months before starting GnRH?

Dr. Santoro. We had one such patient who was 37 years old when she began pulsatile GnRH and had received no other treatment before she started. She ovulated in 17 days, and her uterus showed remarkable growth on pelvic ultrasonography. She did not conceive in her first cycle. Perhaps Dr. Filicori would also like to comment on this.

Dr. Filicori. We have a large population with primary hypothalamic amenorrhea, and we perform ultrasonography at the beginning and during treatment. We do not pretreat these patients with estrogens, and we see the size of the uterus increase about 1 week after the initiation of pulsatile GnRH therapy. As soon as endogenous estrogen levels begin to rise, the uterus will increase in size. We have treated patients with a longitudinal uterine diameter of 5 or 6 cm; by the time they ovulate the uterus is of normal size for an ovulatory woman.

Practical aspects of pulsatile gonadotropin-releasing hormone administration

Eldon D. Schriock, MD
Memphis, Tennessee

Pulsatile administration of gonadotropin-releasing hormone represents a major advance in the treatment of anovulation in women who fail to ovulate with clomiphene citrate and is an alternative for many women who currently receive human menopausal gonadotropin. Four issues must be addressed before administering pulsatile gonadotropin-releasing hormone: (1) safety, (2) efficacy, (3) convenience, and (4) cost. Each of these issues will be affected by the three major decisions a physician makes with gonadotropin-releasing hormone therapy: (1) patient selection, (2) route of administration, and (3) dose of gonadotropin-releasing hormone. The ideal candidate for gonadotropin-releasing hormone therapy is a patient with an absence of endogenous pulsatile gonadotropin-releasing hormone, as seen in hypothalamic amenorrhea. Although women with polycystic ovarian disease can be treated with pulsatile gonadotropin-releasing hormone, a decreased ovulation rate should be expected. The route of administration, intravenous or subcutaneous, and the degree of monitoring can be tailored by the physician to fit each patient's needs. Pulsatile gonadotropin-releasing hormone therapy is a safe, effective, convenient, and economical alternative to human menopausal gonadotropin for ovulation induction in women resistant to clomiphene. (Am J Obstet Gynecol 1990;163:1765-70.)

Key words: Gonadotropin-releasing hormone, hypothalamic amenorrhea, polycystic ovarian disease, intravenous administration of gonadotropin-releasing hormone, subcutaneous administration of gonadotropin-releasing hormone

Pulsatile administration of gonadotropin-releasing hormone (GnRH) represents a major advance in the treatment of anovulation. The use of human menopausal gonadotropin (hMG), clomiphene citrate, and bromocriptine has helped many anovulatory women. Unfortunately, many women remain anovulatory or have side effects as a result of these medications. Pulsatile GnRH therapy is a new option for the treatment of women who fail to ovulate with clomiphene citrate and is an alternative for many women who currently receive hMG.[1,2]

The primary indication for pulsatile GnRH will be ovulation induction in patients who fail to respond to clomiphene citrate. Pulsatile GnRH will be especially advantageous for those patients who live a long distance from a referral center where hMG is available. Because the monitoring required and the potential risks are much less with GnRH than those encountered with hMG, the local physician will be able to administer GnRH. In situations in which hMG is being administered, candidates for pulsatile GnRH will come from those patients now receiving hMG. Today patients treated for infertility undergo ovulation induction for indications other than anovulation. Pulsatile GnRH is appropriate for some, but not all, of these indications. GnRH is appropriate for treating luteal-phase defects and poor cervical mucus and improving timing for therapeutic donor insemination. GnRH is not a good choice to induce ovarian hyperstimulation. Thus GnRH is not an alternative to hMG in those patients undergoing in vitro fertilization and gamete intrafallopian transfer cycles or patients being treated for unexplained infertility.

Pulsatile GnRH can also be used for diagnostic purposes. It can be used to differentiate pituitary from hypothalamic failure. In some patients a single bolus GnRH test will fail to stimulate a gonadotropin response until the pituitary gland has been primed with pulsatile GnRH administration. In rare cases of combined hypothalamic and ovarian failure, pulsatile GnRH therapy will unmask the ovarian failure by allowing gonadotropins to reach menopausal levels during pulsatile GnRH therapy. Because pulsatile GnRH therapy, when administered intravenously, will induce ovulation in nearly 100% of patients with hypothalamic amenorrhea, some investigators believe the diagnosis

From the Department of Obstetrics and Gynecology, University of Tennessee.
Reprint requests: Eldon D. Schriock, MD, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, Room M 1479, San Francisco, CA 94143.
6/0/24544

Table I. Candidates for pulsatile GnRH

Kallmann's syndrome
Tumors
Craniopharyngioma
Trauma
Hypothalamic chronic anovulation
Psychogenic
Nutritional
Exercise related

of hypothalamic amenorrhea must be questioned in those patients who fail to respond to pulsatile GnRH.

The cause of the patient's anovulation should be evaluated thoroughly before GnRH therapy. A standard workup would include assessment of prolactin, thyroid function, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and androgens. Assessment of body mass index and a progestin challenge test can be used to predict the success of GnRH therapy. The minimum infertility workup required would be semen analysis. If the patient has never been exposed to ovulation, tubal function would not need to be evaluated before GnRH therapy. The laparoscopy could be postponed until after initiation of therapy if the patient has no risk factors of tubal disease or endometriosis. It is now common practice to perform a laparoscopy before beginning hMG therapy. This particular practice is less important with pulsatile GnRH therapy, because the risks of GnRH are less than those associated with hMG.

Patient selection

The ideal candidate for GnRH therapy is a patient with an absence of endogenous pulsatile GnRH (e.g., Kallmann's syndrome). These patients respond promptly and successfully to GnRH treatment. Other examples of decreased endogenous GnRH activity are patients with hypothalamic amenorrhea caused by weight loss, exercise, and stress (Table I). Before GnRH therapy, the physician should try to reverse the cause of anovulation, if possible, by decreasing the patient's activity or increasing the patient's weight. Correction of anorexia is critical before GnRH therapy. Women with anorexia respond well to GnRH, but if the anorexia is not corrected the patient's poor diet can result in severe intrauterine growth retardation.[3] Other candidates would be patients who have decreased GnRH function as a result of surgery on hypothalamic-pituitary tumors or those patients who have had trauma to the hypothalamic-pituitary unit. The Food and Drug Administration has given approval for pulsatile GnRH administration only to those patients who carry the diagnosis of primary hypothalamic amenorrhea. Although patients with severe hypothalamic amenorrhea are the ideal candidates for GnRH therapy, milder forms of hypothalamic amenorrhea can also be treated. Patients with normal serum levels of gonadotropins, a positive progestin challenge test result, and normal weight will respond to GnRH but may have decreased rates of success. Mild forms of polycystic ovarian disease will also respond to GnRH therapy. Patients with severe cases of polycystic ovarian disease, including patients with severe obesity (body mass index of greater than 25, markedly elevated androgen levels, or elevated serum LH levels >12 mU/ml), are poor candidates for GnRH therapy.[4] Combined regimens with GnRH agonists may improve success in this group.

Choosing the GnRH alternative

When is it appropriate to put the artificial hypothalamus to work? Each physician must determine if GnRH therapy is practical for his or her office. Four issues used to make this decision are (1) safety, (2) efficacy, (3) convenience, and (4) cost. Pulsatile GnRH is definitely a safer alternative compared with hMG. When used appropriately, there is a marked decrease in the rates of hyperstimulation and multiple births. Although ovarian hyperstimulation, which results in enlarged ovaries with multiple follicles, can occur in GnRH therapy, no reports of ovarian hyperstimulation syndrome have been reported. Most of the multiple births associated with GnRH therapy have resulted from high doses of GnRH or combining the GnRH therapy with clomiphene citrate or human chorionic gonadotropin (hCG) injections. Low-dose GnRH therapy alone results in a very low multiple birth rate. The second risk of GnRH therapy is that of infection. Although phlebitis and pain at the site of intravenous injection are the most commonly reported side effects, no major complications have been reported.[5] The increased use of subcutaneous therapy will decrease this complication further. Although anti-GnRH antibodies have been reported in men and women receiving GnRH therapy, no clinical sequelae have been reported as a result of development of these antibodies.

With proper patient selection, the efficacy of pulsatile GnRH is very high. Patients with severe hypothalamic amenorrhea have an almost 100% ovulatory rate with GnRH. Patient selection is the key factor in determining the success rate of GnRH therapy.

GnRH therapy results in increased convenience for both the physician and the patient. The physician's office benefits from no need for daily monitoring of the patient. This results in a decreased need for weekend staffing. Advantages for the patients include fewer vis-

its to the office and no daily injections, venipunctures, and ultrasound examinations.

The cost to the patient will depend on several factors. The first is route of administration. The increased dose required for subcutaneous administration results in increased drug costs. The duration of therapy is dependent on the patient's diagnosis and route of administration, with subcutaneous therapy taking somewhat longer from onset of therapy to ovulation. The ultimate cost to the patient will be determined by the physician's office charges and the number of GnRH kits required to complete therapy. The largest cost savings resulting from GnRH therapy may be the decrease in multiple births, avoiding the high cost of prenatal and neonatal care for multiple gestations.

Until recently, preparation of the physician's office to administer GnRH was difficult. The equipment and supplies needed to administer GnRH were purchased from multiple vendors unaccustomed to serving the needs of the reproductive endocrinologist. Today kits containing all the essential items are available. The basic items needed for GnRH administration are (1) the pulsatile pump; (2) the set, including the GnRH, tubing, battery, belt to hold the pump, and syringes; (3) heparin for intravenous administration; and (4) an ultrasound machine for monitoring therapy. Because of the ease of GnRH administration, the starting and monitoring of pulsatile GnRH activity can be delegated to a well-trained office nurse. These tasks would include checking the injection site, refilling the pump, and changing the battery.

Before the onset of therapy, the patient must be counseled concerning the benefits and risks. This would include the issues of safety, convenience, and efficacy compared with other available alternatives. The cost should be outlined to the patient and an assessment should be made concerning insurance reimbursement. Time spent on patient education will improve the patient's experience while on the pump and decrease after-hours phone calls. It is ideal to involve the patient's spouse who can then be of help in managing the pump and also understand the purpose of the therapy. It is also helpful to actually show the patient a pump to dispel any misconceptions about the apparatus. If possible, offer the patient the name of a previous patient receiving GnRH with whom she could discuss the therapy.

Method of GnRH administration

A specific method for GnRH administration now has the approval of the Food and Drug Administration. This suggested protocol includes intravenous administration of 5 μg GnRH every 90 minutes, baseline pelvic ultrasonography to be repeated on days 7 and 14 of therapy, changing of the intravenous site every 48 hours, and the administration of GnRH throughout the luteal phase. As with many therapies, for instance hMG, there are many variations and each physician has his or her own individualized regimens. This same individuality and variability has been and is currently practiced by physicians around the world who have administered pulsatile GnRH for the past 10 years.[4]

Table II. Pulsatile GnRH therapy: route of administration

	Intravenous	*Subcutaneous*
Advantages	Greater efficacy	Easy to use
Disadvantages	Infection	Larger dose

Baseline screening immediately before therapy is optional. Appropriate tests are hCG, LH, FSH, estradiol, and a baseline pelvic ultrasound examination. A progestin induction of menses is appropriate in those women who have menstrual bleeding after progestin withdrawal.

The first and most important decision to be made before GnRH therapy will be the route of administration. The controversy over intravenous versus subcutaneous administration stems from the conflicting results with subcutaneous therapy. Initial investigators found decreased success rates with subcutaneous therapy. In spite of these discouraging results, other investigators have reported continued success with subcutaneous therapy, especially when proper patient selection has been performed.[4] Differences in the pharmacokinetics between subcutaneous versus intravenous administration of GnRH have been documented in several studies. Most authors agree that peak levels of GnRH are increased with decreased time to peak and time to return to baseline with intravenous administration compared with subcutaneous administration. In spite of the differences in GnRH concentration when given subcutaneously, clinical studies indicate that in most instances the pituitary gland receives an adequate signal. A major advantage of subcutaneous therapy versus intravenous administration is the decreased risk of infectious complications. Administration of GnRH subcutaneously is much easier, especially in women in whom maintaining an intravenous line is difficult. The decreased dosage of GnRH needed with intravenous therapy may result in a significant economic benefit to patients. Other potential benefits of intravenous treatment are higher ovulatory rates and a more predictable and shorter course of therapy (Table II).

Table III. Lutrepulse for Injection: dose options

Vial (mg)	*Volume/pulse (μl)*	*Dose/pulse (μg)*
0.8	25	2.5
0.8	50	5
3.2	25	10
3.2	50	20

The dose of GnRH per pulse is becoming an increasingly important variable in pulsatile GnRH therapy. Whether a standard dose exists that is appropriate for all women is an unresolved issue. Approximately 75 ng/kg GnRH[7] will stimulate gonadotropin patterns similar to those of normal control subjects. The lowest effective dose has obvious economic advantages and may also be important in controlling the rate of multiple births. Increasing the dose per pulse will increase pregnancy rates and is probably necessary with subcutaneous therapy and in attempts to treat women with polycystic ovarian disease. Few patients ovulate with doses less than 15 μg per pulse during subcutaneous therapy. Women with polycystic ovarian disease have been treated with doses as high as 20 μg per pulse intravenously. The standard dose of administration has become 5 μg per pulse for intravenous therapy and 15 to 20 μg per pulse for subcutaneous therapy. Current packaging has resulted in four dose-per-pulse options including 2.5, 5, 10, and 20 μg per pulse (Table III). To change the dose per pulse, the concentration of GnRH in solution must be changed because the volume per pulse is fixed at either 25 or 50 μl when using Lutrepulse (gonadorelin acetate) for Injection (Ortho Pharmaceutical Corp., Raritan, N.J.) pump. Other pumps are available in which volume per pulse can be regulated.

The ideal goal of GnRH treatment would be to mimic the frequency of normal gonadotropin pulses. Perfect duplication of physiologic pulse frequency is not necessary. The standard pulse frequency is every 90 minutes. Previous studies have shown that therapy only during daytime hours can also be successful. This indicates that brief lapses in pulsatile therapy caused by equipment failure will not result in a significant decrease in efficacy.

Preparation of the pump

A solution of GnRH is made; heparin, 1000 units/ml, is added for intravenous administration. The GnRH solution is placed in a small plastic pouch or syringe that fits inside the pump. Specialized catheter systems are availabe for intravenous delivery, or standard intravenous catheters may be used. Ideally, catheters are placed into a forearm vein by a sterile technique and a surgical-type preparation of the intravenous site. A transparent dressing should be placed over the intravenous site. If placed in this manner, intravenous catheters can be left in place until patient discomfort or early signs of possible phlebitis or extravasation prompt removal with placement of the intravenous line in an alternative site. Specialized infusion sets are also available for subcutaneous administration. Small-gauge needles or a catheter can be used, with infusions most commonly placed into the lower abdominal area. Recent data indicate that placement of the subcutaneous needle in the upper arm at the base of the deltoid muscle may result in increased efficacy.[8] The needle or catheter is repositioned at another site every 3 days by either the physician or the patient.

During therapy, the only physician intervention needed is refilling the pump and checking the site of infusion. The patient can be trained to perform these tasks, especially during subcutaneous therapy. The Lutrepulse for Injection system needs to be refilled and the battery changed weekly. Ultrasound scans can be formed on days 7 and 14, and thereafter the frequency is based on the patient's response. Ultrasound monitoring during the first cycle is helpful in determining the patient's response to therapy and if changes in dose or frequency are indicated. If more than one mature follicle is visualized, it is recommended that GnRH therapy be stopped. Frequency of ultrasound monitoring can be decreased in subsequent cycles once the patient's response becomes predictable. Prediction of ovulation will help the patient time intercourse and can also be used to schedule postcoital tests. Ovulation can be detected with basal body temperature charts, LH-surge detection kits, or ultrasonography. The follicle can be expected to grow approximately 2 mm/day during the late follicular phase. After ovulation, stimulation of the corpus luteum must be maintained.

Pulsatile GnRH therapy can be continued or the pump may be stopped and injections of hCG (1000 to 2500 units intramuscularly every 3 to 4 days) used to support the luteal phase. Preliminary data indicate that continued pulsatile GnRH will result in increased efficacy and will not result in the artificial lengthening of the luteal phase that can be seen with administration of hCG. Most patients will ovulate after 10 to 20 days of GnRH therapy. A delayed response is occasionally seen in initial treatment cycles and may be explained by a need for maturation of pituitary function in women who have never gone through puberty. As therapy is continued, the length of the follicular phase will approach that of a normal cycle. Pulsatile GnRH is

maintained during the luteal phase until documentation of pregnancy by a doubling of the hCG titer or a gestational sac.

Certain questions are commonly asked by patients undergoing GnRH therapy. Patients are often concerned about disturbing the pump during sleep. Before getting into bed, the patient should remove any tape holding the tubing to the stomach or chest while leaving the tubing taped on the arm. The belt with the pump in place is removed and pinned above the pillow to the bed sheet. When bathing or showering, the pump is removed and can be hung outside the shower or tub. Almost any activity can be maintained while the patient is undergoing GnRH therapy. It is difficult to maintain complete privacy while the pump is being used. Although the pump can be covered completely by clothing, the sound of the pump cannot be muffled completely.

Potential problems during GnRH therapy include leaks from the pouch holding the fluid or in the tubing, retrograde flow of blood into the tubing, pain at the injection site, and pump failure. Ideally, a backup pump is present for pump failures, although the patient can self-administer GnRH until a replacement pump is obtained. Patients should be taught how to activate the pump, flush the intravenous line, and remove the intravenous line, in case its emergency removal is necessary. Patients receiving subcutaneous therapy can be taught how to replace the needle if the injection site becomes painful.

Failure to ovulate

In the presence of therapeutic failure, the first step should be to recheck the system and rule out equipment failure or errors in administration. Some patients will respond to an increase in the dose per pulse. Changing pulse frequency from 90 minutes to every 60 minutes may result in some increased efficacy. Some investigators continue to augment pulsatile GnRH therapy with timed hCG administration to facilitate follicular rupture or clomiphene citrate therapy.[9] These methods have been used in patients with mild polycystic ovarian disease who are poor responders to pulsatile GnRH alone. The patients should be aware that the addition of these methods to augment GnRH efficacy will also enhance the rate of multiple births.

Contraindications for pulsatile GnRH therapy

Ovarian and pituitary failure must be ruled out before GnRH therapy. Diagnosis of ovarian failure is determined by the occurrence of elevated gonadotropin levels, except in the rare instance of associated hypothalamic failure. Failure to induce LH and FSH secretion after a single bolus of GnRH is not diagnostic of pituitary failure. The pituitary gland may respond after proper GnRH "priming," which is accomplished by a few days of pulsatile GnRH therapy. Pituitary failure is one of the only disease states in which GnRH therapy is not a possible alternative to hMG. Other contraindications to GnRH therapy include a sensitivity to GnRH or uncorrected anorexia.

In summary, four issues must be addressed before the physician can decide if pulsatile GnRH will be a practical addition to the ovulation-induction regimen in his or her office: (1) safety, (2) efficacy, (3) convenience, and (4) cost. Each of these issues will be affected by the three major decisions a physician makes with GnRH therapy: (1) patient selection, (2) route of administration, and (3) dose of GnRH per pulse. Women with diagnoses other than hypothalamic amenorrhea, including polycystic ovarian disease, may be candidates for pulsatile GnRH therapy, but decreased rates of ovulation should be expected. Subcutaneous administration may prove to be the best route of therapy for initial treatment of women with hypothalamic amenorrhea, with intravenous administration being reserved for unresponsive cases. If women with polycystic ovarian disease are to be treated, GnRH should be given intravenously. Monitoring of therapeutic response with ultrasonography during treatment is needed for increased dosage regimens and in patients suspected of having endogenous GnRH activity. Pulsatile GnRH therapy is a safe, effective, convenient, and economical alternative to hMG for ovulation induction in women resistant to clomiphene.

REFERENCES

1. Schriock ED, Jaffe RB. Induction of ovulation with gonadotropin-releasing hormone. Obstet Gynecol Surv 1986;41:414.
2. Zacur HA. Ovulation induction with gonadotropin-releasing hormone. Fertil Steril 1985;44:435.
3. van der Spuy ZM, Steer PJ, McCusker M, Steele SJ, Jacobs HS. Pregnancy outcome in underweight women following spontaneous and induced ovulation. Br Med J 1988; 296:962.
4. Homburg R, Eshel A, Armar NA, et al. One hundred pregnancies after treatment with pulsatile luteinising hormone releasing hormone to induce ovulation. Br Med J 1989; 298:809.
5. Hopkins CC, Hall JE, Santoro NF, et al. Closed intravenous administration of gonadotropin-releasing hormone: safety of extended peripheral intravenous catheterization. Obstet Gynecol 1989;74:267.
6. Armar NA, Tan SL, Eshel A, et al. Practical aspects of pulsatile LHRH therapy. Br J Hosp Med 1987;5:429.
7. Crowley WF, Filicori M, Spratt DI, Santoro NF. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. Recent Prog Horm Res 1985; 41:473.
8. Blunt SM, Menon BRK, Butt WR. Pulsatile LHRH in hypogonadotrophic hypogonadism. In: Bloom SR, Jacobs

HS, eds. Therapeutic applications of LHRH. London: Royal Society of Medicine Services, 1986:89-98.
9. Braat DDM, Boghelman D, Coelingh-Bennink HJT, et al. The outcome of pregnancies established in GnRH-induced cycles with special reference to multiple pregnancies in five Dutch centres. In: Coelingh-Bennink HGT, Dogterom AA, Lappohn RE, Rolland R, Shoemaker J, eds. Pulsatile GnRH. Haarlem, The Netherlands: Ferring, 1985.

Discussion

Audience. Would you comment on the types of needles and equipment used for both intravenous and subcutaneous delivery of pulsatile GnRH?

Dr. Schriock. For intravenous therapy, you use an angiocatheter of your choice. The pump tubing comes with a connector that will fit in a 20-gauge angiocatheter.

For subcutaneous therapy, you can use an angiocatheter or the needle set that comes with the pump kit. There are also subcutaneous kits that are used for administering insulin to diabetic patients who use insulin pumps.

December **1990**
in two parts, part **1**
volume **163**, *number* **6**
Index number

Acc 12-11-92

177

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editors
ALBERT B. GERBIE
STEVEN G. GABBE
THOMAS J. GARITE
MOON H. KIM
ALBERTO MANETTA



Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS



Published by

MOSBY-YEAR BOOK, INC.
St. Louis, Missouri 63146-3318

ISSN 0002-9378

Going.

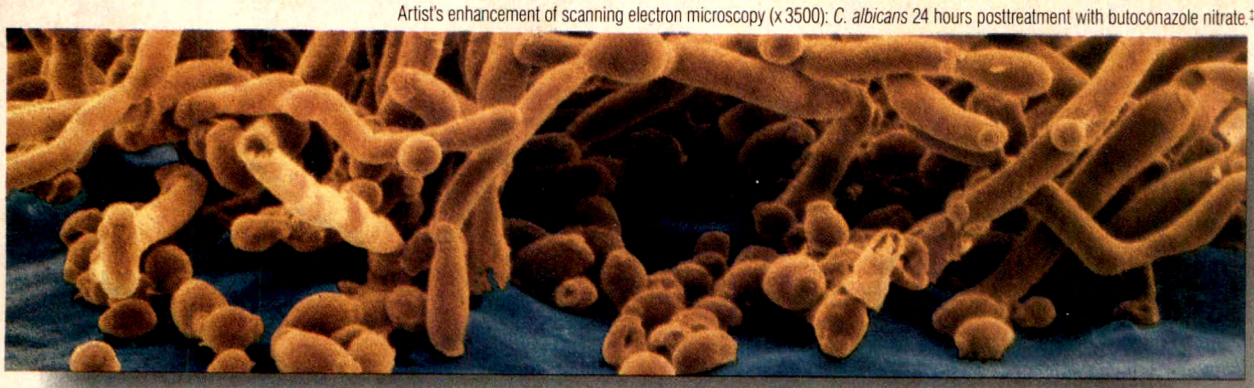
Going.

Gone.

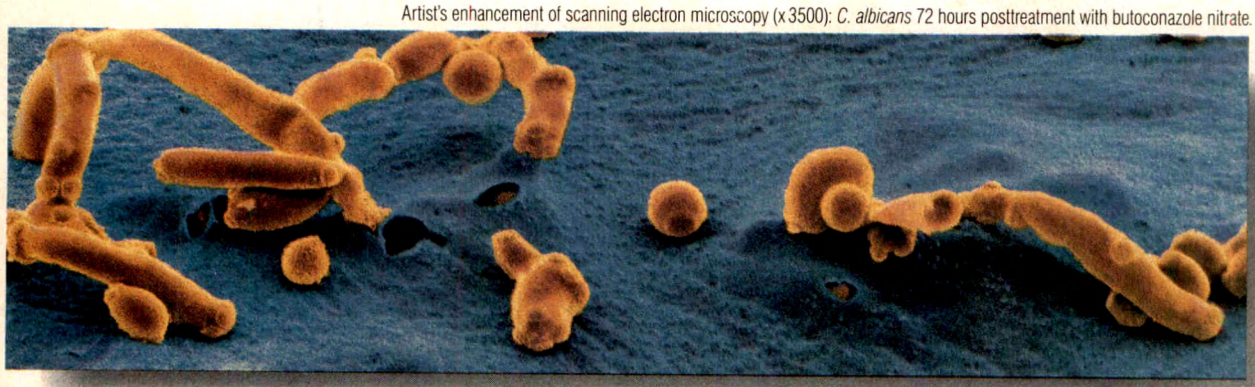
***In vulvovaginal candidiasis,* the proven efficacy of 3-day therapy* with FEMSTAT Prefill means a microbiological cure in 95% of patients.[1†]**

1. Droegemueller W, Adamson DG, Brown D, et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64:530-534. *In pregnant patients (second and third trimesters only), therapy should be extended to six days. †Microbiological cure in 95% of patients (n = 126) eight days following FEMSTAT 3-day therapy. ‡ Micrographs on file, Syntex Laboratories, Inc. Please see next page for brief summary of prescribing information. © 1990 Syntex Laboratories, Inc. 813-H2166-90

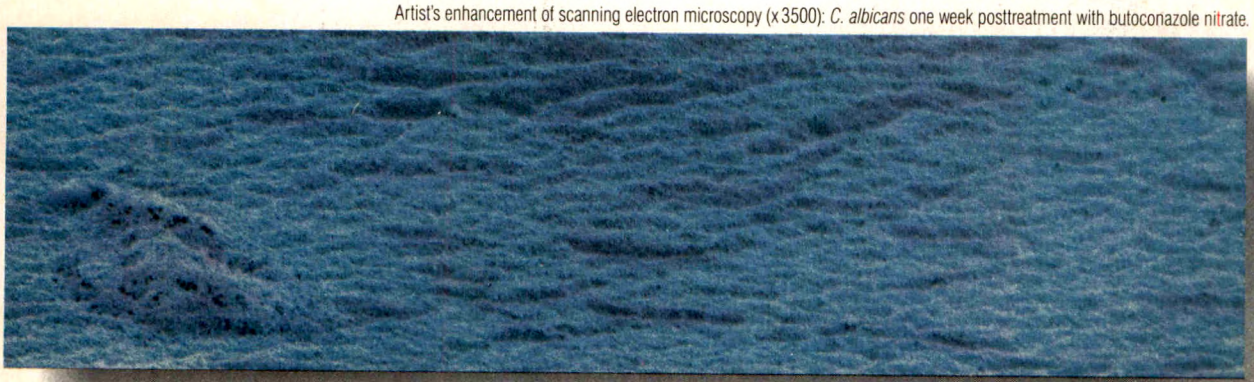
Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 24 hours posttreatment with butoconazole nitrate.‡



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 72 hours posttreatment with butoconazole nitrate.



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* one week posttreatment with butoconazole nitrate.



FEMSTAT® PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%

So effective, it works with 3-day therapy.



SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Committed to the OB/GYN and women's healthcare

FEMSTAT PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

CURRENT PROBLEMS IN OBSTETRICS, GYNECOLOGY AND FERTILITY

Editor-in-Chief: Robert L. Barbieri, MD
State University of
New York at Stony Brook

Current Problems in Obstetrics, Gynecology and Fertility brings you the authoritative, practical information you need to stay current in your specialty and to provide the best possible care to patients.

- Each bimonthly issue provides in-depth, monograph-length coverage (40-60 pages) of a clinical topic of vital interest to practitioners today.
- Topics are selected for relevance to your daily practice: preconceptual counseling, infertility, endocrine problems, high-risk obstetrics, diagnosis and treatment of primary and secondary amenorrhea, pediatric and adolescent gynecology, and more.
- Authors are selected for their expertise; you'll benefit from their balanced perspective on the topic and their wealth of clinical experience.
- Each article is extensively referenced, saving you hours of library research.

Stay at the forefront of your specialty! Subscribe today by returning the order form or by calling our toll-free number.

Yes! Please begin my subscription to ***Current Problems in Obstetrics, Gynecology and Fertility*** (6 issues per year).

	1991 Rates	U.S.A.	Canada	International
☐	Individual	$60.00	$67.00	$85.00*
☐	Student, resident	39.00	46.00	64.00*
☐	Institutional	85.00	92.00	110.00*

* Exclusive of Japan and India. Contact Publisher for rate and agent information.

Individual and student/resident subscriptions must be in the name of, billed to, and paid for by the individual. Please allow 6-8 weeks for receipt of first issue in U.S. Price of international subscriptions includes surface-mail postage.

☐ Send me a custom binder for $20.00. (Holds 12 issues.)

Method of Payment (please check one)

We'll send an extra issue free if payment accompanies your order!

☐ Check (U.S. funds, payable to *Current Problems in Obstetrics, Gynecology and Fertility*)

☐ MasterCard ☐ MC International ☐ VISA ☐ Bill me

Credit card # _____ Exp. date _____

Signature _____

Name _____

Institution _____

Address _____ Apt/Suite/Dept _____

City _____ State _____ ZIP _____

Country _____ Specialty _____

Return to:
Mosby–Year Book, Inc.
Journal Subscription Services
11830 Westline Industrial Drive
St. Louis, MO 63146 U.S.A.

For faster service, phone:
(314) 872-8370, ext. 7351
In the U.S., call toll-free:
1-800-325-4177, ext. 7351
Fax: (314) 432-1380

Mosby Year Book

J5290FZB

Mosby
Year Book

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1990 by Mosby–Year Book, Inc.

Contents

December
Part I
1990

CONSULTANTS

Consultants for the American Journal of Obstetrics and Gynecology 1771

PAPERS OF THE SOCIETY FOR GYNECOLOGIC INVESTIGATION

Is intrauterine transfusion associated with diminished fetal growth? 1781
Gregory O. Utter, MD, Michael L. Socol, MD, Sharon L. Dooley, MD, Scott N. MacGregor, DO, and Dietra D. Millard, MD
Chicago, Illinois

Intrauterine transfusion of anemic, isoimmunized fetuses is not associated with diminished intrauterine fetal growth.

Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus 1785
Dru E. Carlson, MD, Lawrence D. Platt, MD, Arnold L. Medearis, MD, and Janet Horenstein, MD
Los Angeles, California

The biventricular outer dimension of the fetal heart as measured by real-time-directed M-mode echocardiography is the best prognostic indicator in prenatally diagnosed nonimmune hydrops fetalis.

Sources of variability in umbilical artery systolic/diastolic ratios: Implications of the Poiseuille equation 1788
Jeffrey W. Wright, MD, and Louis E. Ridgway, MD
San Antonio, Texas

Term pregnancies were studied to determine the contributions of umbilical cord length, cord blood hematocrit, and fetal heart rate to the variability of systolic/diastolic ratio.

(Contents continued on page 4A)

Vol. 163, No. 6, Part 1, December 1990. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 7351. Annual subscription rates for 1991: domestic, $86.00 for individuals and $150.00 for institutions. Printed in the U.S.A.
Copyright © 1990 by Mosby–Year Book, Inc.



Contents continued from page 3A

Variation and correlation in human fetal umbilical Doppler velocities with fetal breathing: Evidence of the cardiac-placental connection 1792
Julia H. Indik, PhD, and Kathryn L. Reed, MD
Tucson, Arizona

Changes in human fetal umbilical arterial and venous Doppler velocities during fetal breathing provide evidence of the interdependence of umbilical venous and arterial Doppler blood flow velocities and confirm the existance of the Frank-Starling mechanism in the fetal heart.

Potential prenatal predictions of Down syndrome: A statistical analysis 1796
William F. O'Brien, MD, Robert A. Knuppel, MD, MPH, Carol Torres, BS, and Deborah Sternlicht, RN, MS
Tampa, Florida

Potential predictors of Down syndrome, including α-fetoprotein, human chorionic gonadotropin, and biparietal diameter/femur length ratio, are independent measures of relative risk.

The course of labor after endurance exercise during pregnancy 1799
James F. Clapp III, MD
Burlington, Vermont

When well-conditioned women continue a high-intensity running or aerobics program throughout pregnancy, it has a beneficial effect on the course and outcome of labor.

Neonatal morphometrics after endurance exercise during pregnancy 1805
James F. Clapp III, MD, and Eleanor L. Capeless, MD
Burlington, Vermont

When a high-intensity running or aerobics program is continued during pregnancy, it produces an asymmetric pattern of growth restriction that primarily reduces neonatal fat mass.

(Contents continued on page 7A)

1991 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)° Canada	Mexico	Region 1	Region 2	Region 3
Institutions†	$150.00	$182.00	$215.00	$223.00	$238.25	$290.50	$342.50
Individuals‡	$ 86.00	$118.00	$151.00	$159.00	$174.25	$226.50	$278.50
Students, residents‡	$ 40.00	$ 72.00	$105.00	$113.00	$128.25	$180.50	$232.50

Subscription rates include supplements. Single copies are $8.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

°*Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Cefotan arithmetic.

CEFOTAN IV 2g, q12h + doxycycline IV or PO 100 mg, q12h

CENTERS FOR DISEASE CONTROL
•CDC•
RECOMMENDED FOR PID[7]

more cost-effective than cefoxitin[1,2]

less frequent dosing than cefoxitin[3,4]

several times the half-life of cefoxitin[5,6]

single-dose prophylaxis/ twice-daily treatment

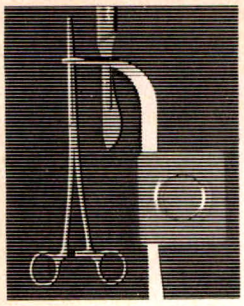


Count on
CEFOTAN® IM/IV
(cefotetan disodium)
In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

© 1989 ICI Americas Inc.



Count on

CEFOTAN® IM/IV
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE
TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:
Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).
Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*
Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*
Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).
Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).
Bone and joint infections caused by *Staphylococcus aureus.**
*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS
CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS
Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS
GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be

developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS
In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.
Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).
Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).
Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).
Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).
Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION
TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED
CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

Manufactured for

STUART PHARMACEUTICALS
A business unit of ICI Americas Inc.
Wilmington, Delaware 19897 USA

Rev. I 11/88 63985-01

Contents continued from page 4A

Does fasting interval affect the glucose challenge test? 1812
Michael D. Berkus, MD, Michael P. Stern, MD, Braxton D. Mitchell, PhD, Edward R. Newton, MD, and Oded Langer, MD
San Antonio, Texas

During pregnancy, the insulin response to a 50 mg glucose challenge is lower when patients have not eaten for ≥3 hours, as compared with a fasting interval of <3 hours.

Relationships between glucose levels and insulin secretion during a glucose challenge test 1818
Michael D. Berkus, MD, Michael P. Stern, MD, Braxton D. Mitchell, PhD, Aida Abashawl, BS, and Oded Langer, MD
San Antonio, Texas

Insulin secretion during a 50 gm glucose challenge test is related to glucose level but not to maternal size, parity, or age.

Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: Results of a controlled, randomized, prospective study 1822
Siri L. Kjos, MD, Donna Shoupe, MD, Sylvie Douyan, MD, Richard L. Friedman, MD, Gerald S. Bernstein, MD, Jorge H. Mestman, MD, and Daniel R. Mishell, Jr., MD
Los Angeles, California

Diabetes developed in women with recent gestational diabetes at a similar rate, and women had comparable lipid profiles at 6 to 13 months whether they were using low-dose oral contraceptives or a nonhormonal birth control method.

Response of placental vasculature to high glucose levels in the isolated human placental cotyledon 1828
Jonathan B. Roth, MD, James A. Thorp, MD, Sue M. Palmer, MD, Peter C. Brath, BS, Scott W. Walsh, PhD, and Sharon S. Crandell, MD
Houston, Texas

Placental vascular resistance increased in response to high levels of glucose in the isolated human placental cotyledon.

Placental thromboxane and prostacyclin production in an ovine diabetic model 1831
Jan E. Dickinson, MD, Bruce A. Meyer, MD, Peter C. Brath, BS, Susie Chmielowiec, MS, Scott W. Walsh, PhD, Valerie M. Parisi, MD, MPH, and Sue M. Palmer, MD
Houston, Texas

Streptozocin-induced ovine diabetes results in a significant reduction in the placental production rate of thromboxane and prostacyclin.

(Contents continued on page 8A)

Contents continued from page 7A

Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns 1836
Naguib A. Samaan, MD, PhD, Pamela N. Schultz, RN, MS, and Frank K. Pham, BS
Houston, Texas

Insulin-like growth factor II appears to play a major role in fetal growth.

Growth factor activity in the blood of women in whom preeclampsia develops is elevated from early pregnancy 1839
Robert N. Taylor, MD, PhD, David C. Heilbron, PhD, and James M. Roberts, MD
San Francisco, California

A prospective, longitudinal cohort study revealed that plasma growth factor activity was elevated significantly in relation to postpartum values, as early as the first trimester, in women in whom preeclampsia eventually developed.

The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term 1844
Michael M. Moretti, MD, Fiona M. Fairlie, MD, Sherif Akl, MD, Aldo D. Khoury, MD, and Baha M. Sibai, MD
Memphis, Tennessee

Oral nifedipine therapy used to reduce blood pressure in patients with preeclampsia remote from term was not associated with any adverse effects on the fetal or uteroplacental circulation as measured by the Doppler technique.

Neuroophthalmologic effects of intravenous magnesium sulfate 1848
Kathleen B. Digre, MD, Michael W. Varner, MD, and Jade S. Schiffman, MD, LTC, MC, USA
Salt Lake City, Utah, and San Francisco, California

Intravenous magnesium sulfate frequently causes visual symptoms and signs.

Low-dose aspirin
II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome 1853
Charles E. L. Brown, MD, Norman F. Gant, MD, Kay Cox, MT(ASCP), Bernard Spitz, MD, Charles R. Rosenfeld, MD, and Ronald R. Magness, PhD
Dallas, Texas

Plasma eicosanoids and angiotensin II pressor responses were measured before and after low-dose aspirin therapy in normal and potentially future hypertensive pregnant women and correlated with pregnancy outcome.

Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat 1861
William C. Mabie, MD, Robert A. Ahokas, PhD, and Baha M. Sibai, MD
Memphis, Tennessee

Captopril effectively lowered maternal blood pressure without adversely affecting organ and uteroplacental perfusion in the hypertensive pregnant rat.

(Contents continued on page 11A)

WE HAVE A POINT TO MAKE.



SO LITTLE CAN DO SO MUCH.

The Smart Start

OVCON® 35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Ovcon® 35 offers your patients contraception, with the lowest norethindrone dose of any combination OC. Low incidence of both androgenic side effects[1] and breakthrough bleeding.[2]

Ovcon 35 has a unique estrogen/progestogen ratio. And remember—Ovcon 35 has no generic equivalent.

Amazing how so little can do so much. Our point. Exactly.

1. Data on file, Bristol-Myers Company.
2. Gordon M, Boria M: Multiclinic study of a low-dose oral contraceptive, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive.* New York, Biomedical Information Corp., 1981, pp 8-11.

Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.

Dedicated to Women's Health Care

Please see next page for brief summary of complete prescribing information.

C-K39-1989

© 1989, Bristol-Myers Company, Evansville, Indiana 47721 U.S.A.

OVCON® 35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptive or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000; ages 35-44, 33/100,000; ages 45-49 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were age 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. (2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledemia; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstibestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) *Gallbladder Disease.* Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Headaches.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. (11) *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) *Breast-Feeding.* A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norephinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein -bound iodine (PBI), T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG, free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, hemolytic uremic syndrome, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erhythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.

C-K39-1989

© 1989, Bristol-Myers Company, Evansville, Indiana 47721 U.S.A.

Contents continued from page 8A

Induction of labor with pulsatile oxytocin 1868
Karen C. Cummiskey, MD, and M. Yusoff Dawood, MD
Louisville, Kentucky, and Houston, Texas

Pulsatile oxytocin required significantly less oxytocin and infusion fluid to induce labor and was as effective as and potentially safer than continuous oxytocin infusion.

Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist 1875
Laird Wilson, Jr., PhD, Michael T. Parsons, MD, and George Flouret, PhD
Chicago, Illinois, and Tampa, Florida

An oxytocin antagonist is a potent inhibitor of spontaneous uterine contractile activity during the last trimester of pregnancy in the baboon.

Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells 1883
Douglas A. Kniss, PhD, John Mershon, MD, Hsing-Chih Su, BS, Jiri Sonek, MD, Richard H. Fertel, PhD, Michael Waxman, BS, Jay D. Iams, MD, and Steven G. Gabbe, MD
Columbus, Ohio

The ubiquitous enzyme protein kinase C potentiates epidermal growth factor–induced prostaglandin E_2 synthesis and may play an important modulatory role in human amnion cell function during the onset of labor.

Lymphokine production during term human pregnancy: Differences between peripheral leukocytes and decidual cells 1890
Donald J. Dudley, MD, Murray D. Mitchell, DPhil, Kathryn Creighton, BS, and D. Ware Branch, MD
Salt Lake City, Utah

Decidual cells, morphologically resembling lymphocytes, produce significantly more granulocyte-macrophage colony-stimulating factor after stimulation than do peripheral leukocytes.

The effect of maternal hyperoxia on behavioral activity in growth-retarded human fetuses 1894
Robert Gagnon, MD, Cora Hunse, RN, and Shubnum Vijan, BSc
London, Ontario, Canada

Maternal hyperoxia produces sustained fetal breathing activity without changes in fetal heart rate or fetal body movement patterns in growth-restricted human fetuses.

Observations on the cause of oligohydramnios in prolonged pregnancy 1900
Kenneth J. Trimmer, MD, Kenneth J. Leveno, MD, Mark T. Peters, MD, and Mary Anne Kelly, RN
Dallas, Texas

Hourly fetal urine production rates were ultrasonographically measured in postterm pregnancies and found to be 50% decreased when oligohydramnios was present.

(Contents continued on page 12A)

Contents continued from page 11A

A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios 1904
Richard Harding, PhD, Stuart B. Hooper, PhD, and Kerry A. Dickson, PhD
Melbourne, Australia

The mechanism whereby oligohydramnios leads to reduced fetal lung expansion and eventual lung hypoplasia was investigated; changes in fetal posture, exacerbated by nonlabor uterine contractions, are considered to play a major role.

Soluble factors produced by isolated first-trimester chorionic villi directly inhibit proliferation of T cells 1914
Richard K. Silver, MD, Jane M. Turbov, BA, Judith A. Beaird, BS, and Joseph Golbus, MD
Evanston, Illinois

Suppression of lymphocyte transformation by chorionic villus culture supernatants suggests that soluble trophoblast factors may contribute to the survival of the fetal allograft.

Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies 1920
David G. Mutch, MD, L. Stewart Massad, MD, Ming-Shian Kao, MD, and John Leslie Collins, PhD
St. Louis, Missouri

Tumor necrosis factor-α and interferon-γ can stimulate or inhibit the in vitro growth of malignant cervical and ovarian cell lines; clinical responses may be similarly heterogeneous.

Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm 1925
Hildur Hardardottir, MD, Tim H. Parmley II, MD, J. Gerald Quirk, Jr., MD, PhD, M. Melinda Sanders, MD, Frank C. Miller, MD, and Timothy J. O'Brien, PhD
Little Rock, Arkansas

Newly developed murine monoclonal antibodies to CA 125 were used to localize antigen in formalin-fixed embryonic tissue, adult apocrine sweat glands, and mammary glands.

Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells 1932
Jay H. Levin, MD, Sharon A. Tonetta, PhD, and Rogerio A. Lobo, MD
Los Angeles, California

Relative potencies of the synthetic progestins norethindrone and medroxyprogesterone acetate compared with natural progesterone, with and without estradiol, for stimulating endometrial stromal cell prolactin production are established.

(Contents continued on page 15A)



"I was devastated when I found out I had genital herpes. What a relief when my doctor recommended a treatment program including Zovirax Ointment."

Zovirax® (acyclovir) Ointment 5% is an ideal complement to any treatment program for initial herpes genitalis. As clinical studies confirm, Zovirax Ointment 5% significantly lessens the duration of pain, shortens viral shedding, *and* speeds healing.[1-3]

Whenever the comfort of a topical antiviral medication is desired, prescribe Zovirax Ointment 5% for initial herpes genitalis. It suits her needs—just when she needs it most.

In initial herpes genitalis,

ZOVIRAX®
(ACYCLOVIR)
OINTMENT 5%
Antiviral Action...
Proven Results

Please see brief summary of prescribing information on next page.

References: **1.** Lacey CJN, Meaden JD, Darville JM, et al. Topical acyclovir ointment in the treatment of genital herpes simplex virus infections. *Eur J Sex Trans Dis.* 1985;2:167-170. **2.** Thin RN, Nabarro JM, Parker JD, Fiddian AP. Topical acyclovir in the treatment of initial genital herpes. *Br J Vener Dis.* 1983;59:116-119. **3.** Corey L, Benedetti JK, Critchlow CW, et al. Double-blind controlled trial of topical acyclovir in genital herpes simplex virus infections. Acyclovir symposium. *Am J Med.* 1982;73:326-334.

ZOVIRAX®
(ACYCLOVIR) OINTMENT 5%

Before prescribing, please consult full package insert, a summary of which follows:

INDICATIONS AND USAGE: Zovirax (Acyclovir) Ointment 5% is indicated in the management of initial herpes genitalis and in limited nonlife-threatening mucocutaneous Herpes simplex virus infections in immunocompromised patients. In clinical trials of initial herpes genitalis, Zovirax Ointment 5% has shown a decrease in healing time and in some cases a decrease in duration of viral shedding and duration of pain. In studies in immunocompromised patients with mainly herpes labialis, there was a decrease in duration of viral shedding and a slight decrease in duration of pain.

By contrast, in studies of recurrent herpes genitalis and of herpes labialis in nonimmunocompromised patients, there was no evidence of clinical benefit; there was some decrease in duration of viral shedding.

Diagnosis: Whereas cutaneous lesions associated with Herpes simplex infections are often characteristic, the finding of multinucleated giant cells in smears prepared from lesion exudate or scrapings may assist in the diagnosis.[1] Positive cultures for Herpes simplex virus offer a reliable means for confirmation of the diagnosis. In genital herpes, appropriate examinations should be performed to rule out other sexually transmitted diseases.

CONTRAINDICATIONS: Zovirax Ointment 5% is contraindicated for patients who develop hypersensitivity or chemical intolerance to the components of the formulation.

WARNINGS: Zovirax Ointment 5% is intended for cutaneous use only and should not be used in the eye.

PRECAUTIONS:

General: The recommended dosage, frequency of applications, and length of treatment should not be exceeded (see DOSAGE AND ADMINISTRATION). There exist no data which demonstrate that the use of Zovirax Ointment 5% will either prevent transmission of infection to other persons or prevent recurrent infections when applied in the absence of signs and symptoms. Zovirax Ointment 5% should not be used for the prevention of recurrent HSV infections. Although clinically significant viral resistance associated with the use of Zovirax Ointment 5% has not been observed, this possibility exists.

Drug Interactions: Clinical experience has identified no interactions resulting from topical or systemic administration of other drugs concomitantly with Zovirax Ointment 5%.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Acyclovir was tested in lifetime bioassays in rats and mice at single daily doses of 50, 150 and 450 mg/kg/day given by gavage. These studies showed no statistically significant difference in the incidence of benign and malignant tumors produced in drug-treated as compared to control animals, nor did acyclovir induce the occurrence of tumors earlier in drug-treated animals as compared to controls. In 2 *in vitro* cell transformation assays, used to provide preliminary assessment of potential oncogenicity in advance of these more definitive lifetime bioassays in rodents, conflicting results were obtained. Acyclovir was positive at the highest dose used in one system and the resulting morphologically transformed cells formed tumors when inoculated into immunosuppressed, syngeneic, weanling mice. Acyclovir was negative in another transformation system.

No chromosome damage was observed at maximum tolerated parenteral doses of 100 mg/kg acyclovir in rats or Chinese hamsters; higher doses of 500 and 1000 mg/kg were clastogenic in Chinese hamsters. In addition, no activity was found in a dominant lethal study in mice. In 9 of 11 microbial and mammalian cell assays, no evidence of mutagenicity was observed. In 2 mammalian cell assays (human lymphocytes and L5178Y mouse lymphoma cells *in vitro*), positive response for mutagenicity and chromosomal damage occurred, but only at concentrations at least 1000 times the plasma levels achieved in man following topical application.

Acyclovir does not impair fertility or reproduction in mice at oral doses up to 450 mg/kg/day or in rats at subcutaneous doses up to 25 mg/kg/day. In rabbits given a high dose of acyclovir (50 mg/kg/day, s.c.), there was a statistically significant decrease in implantation efficiency.

Pregnancy: *Teratogenic Effects.* Pregnancy Category C. Acyclovir was not teratogenic in the mouse (450 mg/kg/day, p.o.), rabbit (50 mg/kg/day, s.c. and i.v.) or in standard tests in the rat (50 mg/kg/day, s.c.). In a non-standard test in rats, fetal abnormalities, such as head and tail anomalies, were observed following subcutaneous administration of acyclovir at very high doses associated with toxicity to the maternal rat. The clinical relevance of these findings is uncertain.[2] There are no adequate and well-controlled studies in pregnant women. Acyclovir should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Zovirax is administered to a nursing woman.

ADVERSE REACTIONS: Because ulcerated genital lesions are characteristically tender and sensitive to any contact or manipulation, patients may experience discomfort upon application of ointment. In the controlled clinical trials, mild pain (including transient burning and stinging) was reported by 103 (28.3%) of 364 patients treated with acyclovir and by 115 (31.1%) of 370 patients treated with placebo; treatment was discontinued in 2 of these patients. Other local reactions among acyclovir-treated patients included pruritus in 15 (4.1%), rash in 1 (0.3%) and vulvitis in 1 (0.3%). Among the placebo-treated patients, pruritus was reported by 17 (4.6%) and rash by 1 (0.3%).

In all studies, there was no significant difference between the drug and placebo group in the rate or type of reported adverse reactions nor were there any differences in abnormal clinical laboratory findings.

OVERDOSAGE: Overdosage by topical application of Zovirax Ointment 5% is unlikely because of limited transcutaneous absorption (see Clinical Pharmacology).

DOSAGE AND ADMINISTRATION: Apply sufficient quantity to adequately cover all lesions every 3 hours 6 times per day for 7 days. The dose size per application will vary depending upon the total lesion area but should approximate a one-half inch ribbon of ointment per 4 square inches of surface area. A finger cot or rubber glove should be used when applying Zovirax to prevent autoinoculation of other body sites and transmission of infection to other persons. **Therapy should be initiated as early as possible following onset of signs and symptoms.**

HOW SUPPLIED: Zovirax Ointment 5% is supplied in 15 g tubes (NDC 0081-0993-94) and 3 g tubes (NDC 0081-0993-41). Each gram contains 50 mg acyclovir in a polyethylene glycol base. Store at 15°-25°C (59°-77°F) in a dry place.

REFERENCES

1. Naib ZM et al. *Cancer Res* 33:1452-1463, 1973.
2. Stahlmann R, Klug S, Lewandowski C, et al. *Infection:* 15(4): 261-262, 1987.

U.S. Patent No. 4199574

IMPROVING LIVES THROUGH ANTIVIRAL RESEARCH

Burroughs Wellcome Co.,
Research Triangle Park,
North Carolina 27709

Copr. © 1989 Burroughs Wellcome Co. All rights reserved.

ZO-1029

Mosby Year Book

MEDICAL PERIODICALS

Dedicated to Publishing Excellence

MEDICAL JOURNALS

American Heart Journal*
American Journal of Infection Control*
American Journal of Obstetrics and Gynecology*
Clinical Pharmacology & Therapeutics*
HEART & LUNG: The Journal of Critical Care*
Hepatology*
The Journal of Allergy and Clinical Immunology*
Journal of the American Academy of Dermatology*
Journal of the American Academy of Physician Assistants
Journal of the American Society of Echocardiography*
Journal of Burn Care & Rehabilitation*
Journal of Child Neurology*
Journal of Geriatric Psychiatry and Neurology*
The Journal of Hand Surgery (American Volume)*
The Journal of Heart and Lung Transplantation*
The Journal of Laboratory and Clinical Medicine*
The Journal of Pediatrics*
The Journal of Thoracic and Cardiovascular Surgery*
Journal of Vascular Surgery*
Otolaryngology—Head and Neck Surgery*
Postgraduate Radiology
Surgery*

CURRENT PROBLEMS SERIES

Current Problems in Cancer*
Current Problems in Cardiology*
Current Problems in Dermatology
Current Problems in Diagnostic Radiology*
Current Problems in Geriatrics
Current Problems in Obstetrics, Gynecology and Fertility
Current Problems in Pediatrics*
Current Problems in Surgery*
Current Problems in Urology
Disease-a-Month*

NEWSLETTERS

Biological Therapies in Psychiatry
Healthline

* Included in Index Medicus

SAMPLES AVAILABLE ON REQUEST J9811FZB

Contact Mosby–Year Book to begin a subscription or for more information on any periodical.

MOSBY–YEAR BOOK, INC.
Journal Subscription Services
11830 Westline Industrial Drive
St. Louis, Missouri 63146 U.S.A.

Call: (314) 872-8370, ext. 7351
In the U.S., call toll-free:
1-800-325-4177, ext. 7351
Fax: 314-432-1380

Contents continued from page 12A

A rabbit model for bacteria-induced preterm pregnancy loss 1938
Raymond A. Dombroski, MD, Donna S. Woodard, PhD, Michael J. K. Harper, PhD, and Ronald S. Gibbs, MD
San Antonio, Texas

Hysteroscopic introduction of bacteria into the uterus of preterm pregnant rabbits leads to pregnancy loss.

In vitro inhibition of esterase activity in amniotic fluid: Comparison with bacterial cultures 1944
Iffath Abbasi Hoskins, MD, Joseph Katz, PhD, Faith J. Frieden, MD, Steven A. Ordorica, MD, and Bruce K. Young, MD
New York, New York

In vitro inhibition of leukocyte esterase activity in amniotic fluid was useful in identifying specific groups of infecting organisms in chorioamnionitis.

Smoking and the antioxidant ascorbic acid: Plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women 1948
Jayasri Basu, PhD, Magdy S. Mikhail, MD, Patricia H. Payraudeau, MD, Prabhudas R. Palan, PhD, and Seymour L. Romney, MD
Bronx, New York

Comparisons were made between healthy smokers and healthy nonsmokers that revealed changes in the levels of ascorbic acid in plasma, leukocytes, and exfoliated cervicovaginal cells.

Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women 1953
Denise Lee Cassidenti, MD, Ariel G. Vijod, MS, Marcela A. Vijod, BS, Frank Z. Stanczyk, PhD, and Rogerio A. Lobo, MD
Los Angeles, California

Smoking induced changes in hepatic estrogen metabolism; this was best reflected by increased serum estrone sulfate and decreased unbound estradiol but normal estradiol and estrone in moderate smokers.

Oxytocin and vasopressin binding sites in human and bovine ovaries 1961
Anna-Riitta Fuchs, DSc, Oliver Behrens, MD, Hanns Helmer, MD, Annette Vangsted, MD, Marina Ivanisevic, MD, Jamie Grifo, MD, PhD, Ciro Barros, PhD, and Michael Fields, PhD
New York, New York, and Gainesville, Florida

Binding sites for oxytocin and vasopressin were found in human and bovine ovaries with cyclic variations that suggest a paracrine role in luteolysis and ovulation.

(Contents continued on page 16A)

Contents continued from page 15A

Correlation of the testicular determinant factor sequence zinc finger Y with varying gonadal phenotypes in a series of 13 subjects with gonadal dysgenesis due to Y aneuploidy 1968

Sandra P.T. Tho, MD, Ali Behzadian, PhD, J. Rogers Byrd, PhD, and Paul G. McDonough, MD

Augusta, Georgia

Zinc finger Y was consistently identified in 4 of 13 individuals with Y aneuploidy and testicular formation; lack of testicular development in the seven remaining zinc finger Y–positive subjects may be due to single-point mutations in zinc finger Y or other pathway genes.

Isolation and characterization of the gene from a human genome encoding 17β-estradiol dehydrogenase: A comparison of Jar and BeWo choriocarcinoma cell lines 1976

Cecil A. Long, MD, Gregory S. Bauer, Mark E. Lowe, MD, PhD, Arnold W. Strauss, MD, and Michael J. Gast, MD, PhD

St. Louis, Missouri

A human gene encoding estradiol dehydrogenase was characterized and expression of this gene in choriocarcinoma cell lines was compared with that of normal cells.

Antiprogestin treatment decreases midluteal luteinizing hormone pulse amplitude and primarily exerts a pituitary inhibition 1982

Donna Shoupe, MD, Daniel R. Mishell, Jr., MD, Gregory Fossum, MD, Bradford L. Bopp, Irving M. Spitz, MD, and Rogerio A. Lobo, MD

Los Angeles, California, and New York, New York

When given in the midluteal phase, RU 486 decreases luteinizing hormone pulse amplitude, possibly through a direct pituitary effect that is not mediated through endogenous opioids.

Luteal insufficiency: Correlation between endometrial dating and integrated progesterone output in clomiphene citrate–induced cycles 1986

Bryan R. Hecht, MD, Wadi A. Bardawil, MD, Firyal S. Khan-Dawood, PhD, and M. Yusoff Dawood, MD

Chicago, Illinois, and Houston, Texas

Clomiphene citrate treatment produced true luteal phase defect as a result of significantly diminished integrated luteal phase progesterone output in only one of 19 cycles.

Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis 1992

Manubai Nagamani, MD, and Charles A. Stuart, MD

Galveston, Texas

In women with polycystic ovarian disease and hyperthecosis, the ovarian stroma has decreased binding sites for insulin but has normal concentration of insulin-like growth factor I receptors.

(Contents continued on page 18A)

LAST YEAR, 4 DOCTORS GAVE UP PRIVATE PRACTICE FOR A CHALLENGING NEW CAREER.



They didn't give up medicine. They just started practicing it in a more exhilarating environment.

An environment that affords them many tangible benefits. Like a diverse practice, unlimited access to medical specialities, and financial security. Plus a lot of intangibles. Like a comfortable lifestyle, an opportunity for worldwide experiences and the respect that goes along with being an Air Force officer.

Of course, to enter an environment this rewarding, they did have to give up a few things. Like rent, equipment expenses, malpractice insurance, payrolls and utility bills.

Now, you may be intrigued by such a unique opportunity. But like any good medical professional, you'd probably like a complete case history. So call 1-800-423-USAF. Or send your curriculum vitae to Colonel William E. Patterson, United States Air Force/RSH, Randolph Air Force Base, Texas 78150-5421.

AIR FORCE

AIM HIGH.

Contents continued from page 16A

Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women 1998
Robert A. Wild, MD, Mary Bartholomew, MS, Deborah Applebaum-Bowden, PhD, Laurence M. Demers, PhD, William Hazzard, MD, and Richard J. Santen, MD
Oklahoma City, Oklahoma, Hershey, Pennsylvania, and Winston-Salem, North Carolina

Sources of hyperandrogenism in hirsute women are heterogeneous as are their lipoprotein lipid profiles; those with high luteinizing hormone/follicle-stimulating hormone ratios may have different reasons for lipid alterations.

Lectins and heparin-binding features of human spermatozoa as analyzed by flow cytometry 2006
Jaime M. Vasquez, MD, Susan F. Magargee, MS, Elaine Kunze, BA, and Roy H. Hammerstedt, PhD
Madison, Wisconsin, and University Park and Philadelphia, Pennsylvania

Flow cytometric analysis of human seminal samples detects unique characteristics of sperm surfaces.

Preembryo biopsy and analysis of blastomeres by in situ hybridization 2013
Jamie A. Grifo, MD, PhD, Ann Boyle, BS, MS, Evan Fischer, BA, Gad Lavy, MD, Alan H. DeCherney, MD, David C. Ward, PhD, and Mrinal K. Sanyal, PhD
New Haven, Connecticut

Blastomeres isolated by preembryo biopsy from mouse and human being were analyzed by in situ hybridization with chromosome- and gene-specific probes.

Factors affecting embryo implantation after human in vitro fertilization: A hypothesis 2020
Richard J. Paulson, MD, Mark V. Sauer, MD, and Rogerio A. Lobo, MD
Los Angeles, California

Pregnancy after human in vitro fertilization is dependent on embryo implantation, which is described as a function of transfer efficiency, embryo quality, and endometrial receptivity.

LETTERS TO THE EDITORS

Prediction of residual trophoblastic tissue in first-trimester abortions: The role of transvaginal Doppler ultrasonography Achiron Reuwen, MD *Jerusalem, Israel* 2024

Suprapubic or vaginal procedure Jaime A. Cabrera, MD, Zsigmund Szekely, MD, and Jairo A. Ospitia, MD *Bogota, Columbia* 2025

Reply Arieh Bergman, MD, Charles A. Ballard, MD, and Paul P. Koonings, MD *Los Angeles, California* 2025

Tubal embryo successfully transferred in utero Landrum B. Shettles, PhD, MD *Las Vegas, Nevada* 2026

Use of midforceps applauded Jorge O. Escamilla, MD *Brooksville, Florida* 2027

Findings not new David A. Abell, MGO, and Normal A. Beischer, MD *East Melbourne, Victoria, Australia* 2027

Reply Oded Langer, MD *San Antonio, Texas* 2028

Contents

Explanation of mechanism of Hyskon solution reaction needed Franklin D. Loffer, MD *Phoenix, Arizona* 2029

Safety of 32% dextran 70 for hysteroscopy Karl-Gösta Ljungström, MD, PhD *Danderyd, Sweden* 2029

Reply R. Jedeikin, MD, and D. Olsfanger, MD *Kjar Saba, Israel* 2029

BOOKS

Books received 2031

ITEMS

Items 2032

INDEX

Author index 2035

Subject index 2060

CORRECTIONS

Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population 2030
(Owen J, Goldenberg RL, Davis RO, Kirk KA, Copper RL. 1990;163:873-9)

The American Board of Obstetrics and Gynecology, Inc. 2030
(1990;162:1645-68)

Transabdominal and transvaginal ultrasonography in the diagnosis of ectopic pregnancy: A comparative study 2030
(Kivikoski AI, Martin CM, Smeltzer JS. 1990;163:123-8)

INFORMATION FOR AUTHORS

Information for Authors 23A

Checklist and copyright statement 28A

Index to advertisers 61A

The JOURNAL is abstracted and/or indexed in *Index Medicus, Science Citation Index, Current Contents/Clinical Medicine, Current Contents/Life Sciences, Excerpta Medica, BRS Colleague,* and MEDLINE.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Mosby–Year Book, Inc., for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page per copy is paid directly to CCC, 27 Congress St., Salem, MA 01970; phone (508)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/90 $00.00 + .10.

The Permanente Medical Group

Northern California



The Permanente Medical Group, the largest multispecialty group practice in the U.S., is undergoing explosive growth in its Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley. Our rapid increase in membership has created practice opportunities throughout the region for additional BC/BE Obstetricians and Gynecologists.

Our physician-managed group is part of the comprehensive Kaiser Permanente Medical Care Program. As a TPMG physician, you have access to the latest medical technology and resources, the support of colleagues in all subspecialties — and the opportunity to provide excellent health care without the burdens of managing a practice.

Join us in one of these facilities:

Quality

Stability

Leadership

Growth

Support

Rewards

Richmond — Small, congenial clinic setting staffed by five physicians and five nurse practitioners with ready consultation for other services, including perinatology. Practice general OB/GYN, high-risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser welcome. Resident teaching opportunities exist. Staff call at Kaiser Hospital in Oakland, shared with resident staff.

Oakland — Twenty physicians, 12 residents offer services in oncology, infertility, maternal-fetal medicine and a large ICN.

Redwood City — Specializes in family-centered maternity care; offers modern LDR rooms and full range of gynecological surgery. With ten physicians, 24-hour call is required three times each month. Beautiful Peninsula location, near San Francisco and the Pacific Ocean.

Stockton — Small four physician department in a new clinic building with new equipment, including laser. Stockton's lifestyle is affordable and attractive, offering recreational activities such as boating on the inland waterways. Centrally located, with mountains to the east and San Francisco Bay Area to the west.

Roseville — Eight physicians and two nurse practitioners in clinic setting. Patients are hospitalized for delivery and surgery in Sacramento where physicians take call for L&D and emergency about 5 times each month.

San Francisco — Full range of services including large high-risk, tertiary care OB service. Eighteen physicians, including GYN Oncologist. Residency program. Clinical research and academic teaching appointments available.

Sacramento — Growing metropolitan area between San Francisco Bay Area and Sierra Nevada mountains. Up-to-date facility with active staff of 14 and excellent residency program affiliated with the University of California at Davis. Flexible call averaging five shifts per month covers L&D, emergency room and inpatient services.

Pleasanton — Staffed by four physicians and three nurse practitioners, clinic provides general OB/GYN services, non-stress tests, ultrasound and cystoscopy. Consultant support, including a Perinatologist, readily available. Share call with staff of Walnut Creek Medical Center. Affiliated with the University of California at San Francisco as clinical instructors.

Fresno — Ground-floor opportunities in growing area for clinically-oriented individuals. Provide general OB/GYN; special interests encouraged. Planning new $26 million outpatient facility to accompany current medical office building. On call every fourth night and weekend. Local residency program at Valley Medical Center affiliated with the University of California at San Francisco. Teaching positions possible.

South San Francisco — Full service except L&D. Nine physicians, six nurse practitioners. On San Francisco Peninsula, 15 minutes from downtown.

TPMG offers many benefits: scheduled time off with cross-coverage provided by your colleagues, continuing education, malpractice insurance, a substantial retirement program and special arrangements for physicians transferring from established practice. For complete information, call or send CV to: The Permanente Medical Group, Inc., Richmond Prescott, M.D., Physician Recruitment Services, Dept. AOG-8348, 1814 Franklin, 4th Floor, Oakland, CA 94612. (800) 777-4912. EOE

KAISER PERMANENTE
Good People. Good Medicine.

American Journal of Obstetrics and Gynecology

Editors

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*
FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*
E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*
STEVEN G. GABBE, *Associate Editor*
THOMAS J. GARITE, *Associate Editor*
MOON H. KIM, *Associate Editor*
ALBERTO MANETTA, *Associate Editor*

Advisory committee on policy

Garland Anderson	Walter S. Keifer	Frank Miller
William R. Anderson	M. Tucker Laffitte, Jr.	Daniel R. Mishell, Jr.
Mason C. Andrews	Emmet J. Lamb	John C. Morrison
E. Forrest Boyd	Roger A. Lobo	Karl C. Podratz
Robert C. Cefalo	James M. Maharry	Ruth W. Schwartz
Donald R. Coustan	L. Russell Malinak	James R. Scott
A. Jerome Freeland	Douglas J. Marchant	Henry A. Thiede
Norman F. Gant	Arthur M. McCausland	Carol Trumbold
W. Glenn Hurt	James A. Merrill	Ronald V. Wade

Board of corresponding editors

Luigi Carenza, *Rome, Italy*	Nils-Otto Sjöberg, *Malmö, Sweden*
T. K. A. B. Eskes, *Nijmegen, The Netherlands*	Gordon M. Stirrat, *Bristol, England*
Wolfgang Künzel, *Giessen, West Germany*	Claude Sureau, *Paris, France*
Matsuto Mochizuki, *Kobe, Japan*	Carl Wood, *Melbourne, Victoria, Australia*
M. Reuben Peyser, *Tel Aviv, Israel*	Olavi Ylikorkala, *Helsinki, Finland*

Publisher
MOSBY-YEAR BOOK, INC., 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*



American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover the Journal is the official publication of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY

GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

CLEVELAND SOCIETY OF OBSTETRICIANS AND GYNECOLOGISTS

American Journal of Obstetrics and Gynecology

Copyright © 1990 by Mosby–Year Book, Inc.

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
Steven G. Gabbe, *Associate Editor*
Moon H. Kim, *Associate Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
Thomas J. Garite, *Associate Editor*
Alberto Manetta, *Associate Editor*
University of California, Irvine, Medical Center,
Department of Obstetrics and Gynecology, 101 The
City Drive, South, Building 26, Orange, CA 92668

Sherry A. Merryman, *Editorial Assistant*
Tel.: (714) 634-6572

Publisher
MOSBY-YEAR BOOK, INC., 11830 Westline
Industrial Drive, St. Louis, Missouri 63146-3318

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370 (ext. 7591)

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. Donald R. Coustan, Department of Obstetrics and Gynecology, Women and Infants Hospital of Rhode Island, 101 Dudley St., Providence, RI 02905.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. The specified copyright statement that follows the Information for Authors in each issue of the JOURNAL must be completed, signed by all authors, and submitted with the manuscript. (The Checklist and Copyright Statement may be torn from the JOURNAL or photocopied for submission.) If not completed in full, it will be returned to the author for completion.

Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the

Editors that manuscripts emanating from a particular institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed. The species of nonhuman animals used in an investigation must be named in the title, abstract, and key words of the manuscript.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

Disclaimer. Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus.*

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name,* and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

For *black-and-white illustrations* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy photographic prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black-and-white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted.

Do not use paper clips or mar the surface of prints in any way.

Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with): author(s) name(s), title of article, number, and top marked clearly.

Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in

sequence after the references. Titles should be included in the legend, *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Letters to the Editors. Letters to the Editors must be submitted in duplicate, typed double-spaced, and the text of the manuscript must be limited to a maximum of 400 words [excluding references, name(s) and address(es) of the signer(s), and the phrase "To the Editors"]. The submitted Letter to the Editors must be accompanied by a signed copyright statement. If there are two or more signers of the Letter to the Editors, one of the authors may sign the copyright statement but must add to the statement the following: "I sign for and accept responsibility for releasing this letter on behalf of any and all co-signers." These requirements apply to all types of Letters to the Editors. Letters should be sent to Dr. Zuspan.

Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints

Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

CHECKLIST AND COPYRIGHT STATEMENT

Both the checklist and the copyright statement forms **must be completed** and sent with each submitted manuscript. If **not** completed, these forms will be returned to the author for completion.

These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

—The original and two copies of the manuscript are submitted.
—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.
—Three sets of unmounted glossy photographic prints are submitted, properly numbered and labeled on the back of each print.
—Three sets of computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back are submitted. None are dot matrix or photographic prints.
—Human experimentation has been approved by the local institution as stated in the Methods section.
—Guidelines for the care and use of animals approved by the institution have been followed as indicated in the Methods section.
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
—All pages are numbered in the following order: title page, condensation, abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
—Signed, written permission from both the copyright holder and the original author for the use of tables, figures, or quotations previously published and their complete references are enclosed with the manuscript.
—Signed, written permission for the use of quotations of personal communications and unpublished data has been obtained from the person(s) being quoted and is enclosed.

Authorship

—In the covering letter that accompanies the submitted manuscript I/we have confirmed that all authors fulfilled both conditions required for authorship.

Conflict of interest

—In the covering letter that accompanies the submitted manuscript the commercial association of the author or of any co-authors that might pose a conflict of interest is described.

Previous publications

—Enclosed with the submitted manuscript are two reprints each of articles the author or co-authors have previously published or have in manuscript form dealing with same patients, same animals, same laboratory experiment, or same data, in part or in full, as those reported in the submitted manuscript. Further explanation is provided in the covering letter that accompanies the submitted manuscript.

Title page

—These elements are given in the following sequence, and are typed double-spaced.
—Title
—Author(s) name(s) and highest academic degree(s)
—City(ies), state(s), and country other than U.S. in which the study was conducted are given
—The name(s) of the institution(s) section(s), division(s), department(s) in which the study was performed is provided and the author(s) institutional affiliation at the time of the study is indicated
—Acknowledgment of financial support is cited
—Name, address, and business and home telephone numbers of author to whom requests for reprints are to be sent
—Reprints will not be available. This has been stated on the title page
—Since the corresponding author is different from the author to whom reprint requests are to be sent, his/her name, address, and business and home telephone numbers have been added
—At the bottom of the page, a running head not exceeding 52 characters (including word spaces) has been typed

Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract

—The abstract is typed double-spaced with required margins (on page 3) headed by the title and the author(s) name, not exceeding 150 words. For case reports and brief communications the limit of 50 words is not exceeded.
—Three to five key words or short phrases are placed beneath the abstract on page 3.

Tables

—Each table headed by a caption and numbered in Roman numerals is typed double-spaced on a separate page.
—Tables are cited in sequence in the text.

Figures

—Are numbered in Arabic numerals and are cited in numerical sequence in the text.

—Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

References

—Are typed double-spaced.

—Are numbered consecutively in the order they are cited in the text.

—The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors were followed.

—Abstracts, personal communications, and unpublished work are not used as numbered references but are mentioned in the text with the written approval of the person being quoted and the signed approval is enclosed.

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

COPYRIGHT STATEMENT

Must be signed by ALL authors

"The undersigned author(s) transfers all copyright ownership of the manuscript entitled

to Mosby–Year Book, Inc., in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author."

The responsible author(s) must be named_____ _____
(Type name) Signature

Each author's name must be typed underneath the signature.

_____ _____

_____ _____

_____ _____

_____ _____

Date: _____

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 163 *number* 6 *part* 1 DECEMBER 1990

CONSULTANTS

Consultants for the American Journal of Obstetrics and Gynecology

September 1, 1989—August 31, 1990

The Editors and Mosby–Year Book, Inc., take this opportunity to acknowledge those reviewers who have contributed many hours and much effort in the evaluation of manuscripts submitted to the JOURNAL during the past year. Without their interest and help, publication of the JOURNAL would be very difficult indeed. We also continue to seek advice from reviewers and readers alike as regards their overall evaluation of the JOURNAL.

Abraham, Guy, *Torrance, California*
Abrams, Robert M., *Gainesville, Florida*
Acker, David B., *Newton Center, Massachusetts*
Aikawa, Masamichi, *Cleveland, Ohio*
Aksel, Sezer, *Mobile, Alabama*
Albrecht, Eugene D., *Baltimore, Maryland*
Allen, Hugh, *Columbus, Ohio*
Allen, Richard, *Portland, Oregon*
Altshuler, Geoffrey, *Oklahoma City, Oklahoma*
Altura, B.M., *Brooklyn, New York*
Alvarez, Manuel, *New York, New York*
Amstey, Marvin S., *Rochester, New York*
Anderson, Barrie, T., *Iowa City, Iowa*
Anderson, Garland D., *Galveston, Texas*
Anderson, Jean, *Baltimore, Maryland*
Anderson, Stephen G., *Winston-Salem, North Carolina*
Andrews, Mason C., *Norfolk, Virginia*
Ansbacher, Rudi, *Ann Arbor, Michigan*
Anthony, F., *Southampton, England*
Apuzzio, Joseph J., *Newark, New Jersey*
Argyrigos, Theofilopolous, *La Jolla, California*
Arias, Fernando, *St. Louis, Missouri*
Artal, Raul, *Los Angeles, California*
Ary, James P., *Laguna Niguel, California*
Asch, Ricardol H., *Garden Grove, California*
Asrat, Tamerou, *Orange, California*
Aubry, Richard H., *Syracuse, New York*
August, Phyllis, *New York, New York*
Auletta, Frederick J., *Burlington, Vermont*
Austin, H., *Birmingham, Alabama*
Azumi, Norio, *Washington, D.C.*
Baggish, Michael S., *Syracuse, New York*
Baker, Carol J., *Houston, Texas*
Baker, David A., *Stony Brook, New York*
Baker, Vicki, *Birmingham, Alabama*
Ballard, Charles A., *Los Angeles, California*
Balmaceda, Jose P., *Orange, California*
Bamforth, J. Stephen, *Edmonton, Alberta, Canada*
Barber, Hugh R.K., *New York, New York*
Barbieri, Robert, *Boston, Massachusetts*
Barden, Tom P., *Cincinnati, Ohio*
Barnes, Randall B., *Chicago, Illinois*
Barron, Donald H., *Gainesville, Florida*
Barron, William, *Chicago, Illinois*
Barss, Vanessa, *Boston, Masschusetts*
Bassell, Gerry, *Wichita, Kansas*
Bast, Robert, *Durham, North Carolina*
Battaglia, Frederick D., *Denver, Colorado*
Baylis, C., *Morgantown, West Virginia*
Beard, Richard W., *London, England*
Beck, Peter, *Edmonton, Alberta, Canada*
Beckmann, Anna Marie, *Seattle, Washington*
Beer, Alan E., *North Chicago, Illinois*
Beller, Fritz K., *Iowa City, Iowa*
Benacerraf, Beryl, *Boston, Massachusetts*
Benedetti, Thomas J., *Seattle, Washington*
Benirschke, Kurt, *La Jolla, California*
Benson, Ralph C., *Portland, Oregon*
Bent, Alfred E., *Long Beach, California*
Berchuck, Andrew, *Durham, North Carolina*
Berek, Jonathan S., *Los Angeles, California*
Bergman, Arieh, *Los Angeles, California*
Berkowitz, Richard, *New York, New York*
Berkowitz, Ross S., *Boston, Massachusetts*
Berland, L.L., *Birmingham, Alabama*
Berman, Michael, *Orange, California*
Bernal, Jose M., *Santander, Spain*
Bhatia, Narender N., *Torrance, California*
Bibbo, Marluce, *Chicago, Illinois*
Bischof, Paul A., *Geneva, Switzerland*
Bissonnette, John M., *Portland, Oregon*
Blackwell, Richard E., *Birmingham, Alabama*

Blakemore, Karin, *Baltimore, Maryland*
Blanchette, Howard A., *Berkeley, California*
Blank, Marian A., *Brooklyn, New York*
Blennerhassett, Michael G., *Hamilton, Ontario, Canada*
Bloomer, William, *Pittsburgh, Pennsylvania*
Blumenfeld, Michael, *Columbus, Ohio*
Bobitt, John R., *El Paso, Texas*
Bocking, Alan, *London, Ontario, Canada*
Boehm, Frank H., *Nashville, Tennessee*
Bolognese, Ronald J., *Philadelphia, Pennsylvania*
Bonnar, John, *Dublin, Ireland*
Boronow, Richard C., *Jackson, Mississippi*
Bottoms, Sidney F., *Detroit, Michigan*
Bowen, Larry, *Sacramento, California*
Bowes, Watson, Jr., *Chapel Hill, North Carolina*
Bowie, James D., *Durham, North Carolina*
Bowman, John, *Winnipeg, Manitoba, Canada*
Boyer, K.M., *Chicago, Illinois*
Brace, Robert A., *La Jolla, California*
Bracken, Michael, *New Haven, Connecticut*
Bradshaw, Ralph A., *Irvine, California*
Braly, Patricia S., *San Diego, California*
Brame, Robert G., *Greenville, North Carolina*
Branch, D. Ware, Jr., *Salt Lake City, Utah*
Brans, Yves W., *Detroit, Michigan*
Braunstein, G.D., *Los Angeles, California*
Braverman, Irwin, *New Haven, Connecticut*
Breen, James L., *Livingston, New Jersey*
Brenner, Paul, *Los Angeles, California*
Brewer, John I., *Chicago, Illinois*
Broadman, Lynn, *Washington, D.C.*
Brodsky, Michael, *Orange, California*
Bronson, Richard A., *Manhasset, New York*
Brosens, I.A., *Leuven, Belgium*
Broughton-Pipkin, Fiona, *Nottingham, England*
Brown, Martin, *Stanford, California*
Brown, Zane A., *Seattle, Washington*
Brumfield, Cynthia G., *Birmingham, Alabama*
Bruner, Joseph P., *Nashville, Tennessee*
Brzyski, Robert, *Norfolk, Virginia*
Bump, Richard C., *Richmond, Virginia*
Burk, Billy D., *Rome, Georgia*
Burrow, Gerard, *La Jolla, California*
Burry, Kenneth A., *Portland, Oregon*
Burton, Barbara, *Chicago, Illinois*
Buster, John E., *Memphis, Tennessee*
Butler, William J., *Albany, New York*
Buttram, Veasy C., Jr., *Houston, Texas*
Cabaniss, Micki S., *Mobile, Alabama*
Campbell, Winston A., *Farmington, Connecticut*
Cantrell, Cathy, *San Diego, California*
Capeless, Eleanor L., *Burlington, Vermont*
Caritis, Steven N., *Pittsburgh, Pennsylvania*
Carlson, Dru E., *Los Angeles, California*
Carpenter, Marshall W., *Providence, Rhode Island*
Carpenter, Robert J., Jr., *Houston, Texas*
Carr, Bruce R., *Dallas, Texas*
Carr, Stephen, *Providence, Rhode Island*
Carsten, Mary, *Los Angeles, California*
Casey, M. Linette, *Dallas, Texas*
Castillo, Ramon A., *Augusta, Georgia*
Castro, Lony, *Los Angeles, California*
Catalano, Patrick M., *Cleveland, Ohio*
Cates, W., *Atlanta, Georgia*
Cavanagh, Denis, *Tampa, Florida*
Cefalo, Robert C., *Chapel Hill, North Carolina*
Cesario, Thomas, *Orange, California*
Cetrulo, Curtis L., *Boston, Massachusetts*
Chakravarti, Aravinda, *Pittsburgh, Pennsylvania*
Challis, John, *London, Ontario, Canada*
Chalmers, Beverley, *River Club, South Africa*
Chang, R. Jeffrey, *Sacramento, California*
Chard, Tim, *London, England*
Charles, Allan, *Chicago, Illinois*
Chasnoff, Ira Jay, *Chicago, Illinois*
Chatterton, Robert, *Chicago, Illinois*
Chaudhuri, Gautam, *Los Angeles, California*
Chervenak, Frank, *New York, New York*
Chesley, Leon C., *Valley Stream, New York*
Chesney, Russel W., *Memphis, Tennessee*
Chestnut, David H., *Iowa City, Iowa*
Cheung, Cecilia Y., *La Jolla, California*
Chez, Ronald A., *Tampa, Florida*
Chitkara, Usha, *New York, New York*
Christopherson, William, *Louisville, Kentucky*
Cibils, Luis A., *Chicago, Illinois*
Clapp, James Ford, III, *Cleveland, Ohio*
Clark, Kenneth, *Cincinnati, Ohio*
Clark, Robin, *Loma Linda, California*
Clark, Stephen L., *Provo, Utah*
Clarke, D.W., *Kingston, Ontario, Canada*
Clarke, G.N., *Carlton, Victoria, Canada*
Clayton, A.L., Beckenham, *Kent, England*
Cleary, Robert E., *Indianapolis, Indiana*
Clewell, William H., *Phoenix, Arizona*
Cohen, Arnold W., *Philadelphia, Pennsylvania*
Cohen, Carmel J., *New York, New York*
Cohen, Jacque, *New York, New York*
Collea, Joseph V., *Potomac, Maryland*
Collins, John, *Hamilton, Ontario, Canada*
Collins, John L., *St. Louis, Missouri*
Comerota, A.J., *Philadelphia, Pennsylvania*
Comstock, Christine H., *Royal Oak, Michigan*
Cooper, J.E., *Nottingham, England*
Copel, Joshua, *New Haven, Connecticut*
Copeland, Larry J., *Columbus, Ohio*
Copeland, William E., Jr., *Columbus, Ohio*
Cordero, Leandro, *Columbus, Ohio*
Cotton, David B., *Houston, Texas*
Coulam, Carolyn B., *Indianapolis, Indiana*
Cousins, Larry M., *Loma Linda, California*
Coustan, Donald R., *Providence, Rhode Island*
Cowchock, Susan, *Philadelphia, Pennsylvania*
Cox, Susan, *Dallas, Texas*
Craig, Robert, *Chicago, Illinois*
Crane, James P., *St. Louis, Missouri*
Creasman, William T., *Charleston, South Carolina*
Creasy, Robert K., *Houston, Texas*
Crenshaw, Carlyle, *Baltimore, Maryland*
Crombleholme, William R., *San Francisco, California*

Crosby, Warren M., *Oklahoma City, Oklahoma*
Cruikshank, Dwight, *Salt Lake City, Utah*
Cruikshank, Stephen H., *Minneapolis, Minnesota*
Cruz, Amelia C., *Gainesville, Florida*
Cullen, Mark T., *Jacksonville, Florida*
Cunningham, F. Gary, *Dallas, Texas*
Cunningham, Jerome, *Columbus, Ohio*
Curet, Luis B., *Albuquerque, New Mexico*
Curran, James, *Atlanta, Georgia*
Curry, Cynthia, *Fresno, California*
Curry, Stephen L., *Hartford, Connecticut*
Curry,Thomas, *Lexington, Kentucky*
Curtin, John P., *Los Angeles, California*
D'Alton, Mary E., *Boston, Massachusetts*
D'Ercole, A. Joseph, *Chapel Hill, North Carolina*
Daffos, Fernand, *Paris, France*
Daly,Douglas, *Worcester, Massachusetts*
Daniell, James F., *Nashville, Tennessee*
Das, Chandra, *Bethesda, Maryland*
Davajan, Val, *Los Angeles, California*
Davidson, Ezra C., Jr., *Los Angeles, California*
Davis, Ann, *Canton, Massachusetts*
Davis, Owen K., *New York, New York*
Davis, Richard, *Birmingham, Alabama*
Davison, John M., *Newcastle-upon-Tyne, England*
Dawes, Geoffrey, *Oxford, England*
Dawood, Mohamed Yusoff, *Houston, Texas*
Dawson, M. Joan, *Urbana, Illinois*
De Prosse, Charles, *Iowa City, Iowa*
DeCherney, Alan H., *New Haven, Connecticut*
DeGroot, Leslie, *Chicago, Illinois*
DePetrillo, Amodio, *Toronto, Ontario, Canada*
DeVoe, Larry, *Augusta, Georgia*
DeVore, Greg, *Salt Lake City, Utah*
Deddish, Ruth, *Chicago, Illinois*
Delivoria-Papadopoulos, Maria, *Philadelphia, Pennsylvania*
Deter, Russell L., *Houston, Texas*
DiLauro, Sharon, *Augusta, Georgia*
DiSaia, Philip J., *Orange, California*
DiZerega, Gere S., *Los Angeles, California*
Diamond, Michael P., *New Haven, Connecticut*
Dickey, Jeffe, *St. Louis, Missouri*
Dierker, Le Roy, Jr., *Cleveland, Ohio*
Dignam, William J., *Los Angeles, California*
Dillman, Robert E., *Newport Beach, California*
Dilts, Preston V., Jr., *Ann Arbor, Michigan*
Divers, Walter A., *San Francisco, California*
Divon, Michael, *Bronx, New York*
Dmowski, W. Paul, *Chicago, Illinois*
Dodds, Wiliam, *Columbus, Ohio*
Dodson, William C., *Hershey, Pennsylvania*
Donnenfeld, Alvin M., *New York, New York*
Dooley, Sharon L., *Chicago, Illinois*
Farmakides, George B., *Mineola, New York*
Farmer, Richard, *Los Angeles, California*
Faulk, Pagie, *Indianapolis, Indiana*
Felig, Philip, *New York, New York*
Ferenczy, Alex, *Montreal, Quebec, Canada*
Ferguson, James, *Charlottesville, Virginia*
Ferin, Michael J., *New York, New York*
Ferris, Thomas J., *Minneapolis, Minnesota*
Figueroa, Jorge P., *Ithaca, New York*
Filly, R.A., *San Francisco, California*
Fishburne, John, *Oklahoma City, Oklahoma*
Fleischer, Adiel, *New Hyde Park, New York*
Fletcher, Mary Ann, *Washington, D.C.*
Flickinger, George L., Jr., *Philadelphia, Pennsylvania*
Foley, Mike, *Phoenix, Arizona*
Fortunato, Stephen J., *Johnson City, Tennessee*
Fotherby Ken, *London, England*
Fowler, Wesley C., Jr., *Chapel Hill, North Carolina*
Fox, Harold E., *New York, New York*
Fraser, H.M., *Edinburgh, Scotland*
Freeman, Ellen, *Philadelphia, Pennsylvania*
Freeman, H.E., *Los Angeles, California*
Freeman, Roger K., *Long Beach, California*
Friedman, Andrew J., *Boston, Massachusetts*
Friedman, Chad I., *Columbus, Ohio*
Friedman, Emanuel A., *Boston, Massachusetts*
Frigoletto, Frederick D., *Boston, Massachusetts*
Fuchs, Anna-Riita, *New York, New York*
Fuchs, Fritz, *New York, New York*
Fuchs, P.G., *Erlangen, West Germany*
Gabbe, Steven G., *Columbus, Ohio*
Douglas, Jessie T., *Glasgow, Scotland*
Driscoll, Shirley G., *Boston, Massachusetts*
Droegemueller, William, *Chapel Hill, North Carolina*
Druzin, Maurice L., *New York, New York*
Ducsay, Charles A., *Loma Linda, California*
Duff, W. Patrick, *Gainesville, Florida*
Dunaif, Andrea, *New York, New York*
Dunn, Randy, *Houston, Texas*
Dyson, Donald C., *Santa Clara, California*
Easterling, Thomas Robert, *Seattle, Washington*
Eckfeldt, John, *Minneapolis, Minnesota*
Edelstone, Daniel I., *Pittsburgh, Pennsylvania*
Ehrlich, Clarence E., *Indianapolis, Indiana*
Eisenberg, Shlomo, *Jerusalem, Israel*
Elias, Sherman, *Memphis, Tennessee*
Elkins, Thomas E., *Ann Arbor, Michigan*
Elliott, John P., *Scottsdale, Arizona*
Enhorning, G., *Buffalo, New York*
Enkin, Murray, *Hamilton, Ontario, Canada*
Entman, Steven *Nashville, Tennessee*
Erickson, Gregory F., *La Jolla, California*
Eschenbach, David A., *Seattle, Washington*
Ettinger, Bruce, *San Francisco, California*
Evans, Alfred, *New Haven, Connecticut*
Evans, Arthur T., *Sacramento, California*
Evans, Mark I., *Detroit, Michigan*
Fadel, Hossam, E., *Augusta, Georgia*
Fainstat, Theodore, *San Jose, California*
Falch, Jan A., *Oslo, Norway*
Fanaroff, Avery, *Cleveland, Ohio*
Fantl, J. Andrew, *Richmond, Virginia*
Gabert, Harvey A., *New Orleans, Louisiana*
Gagnon, Maurice R., *Ontario, Canada*
Galask, Rudolph P., *Iowa City, Iowa*
Gall, Stanley, *Chicago, Illinois*

Gallagher, J. Christopher, *Omaha, Nebraska*
Gallup, Donald George, *Augusta, Georgia*
Gambrell, Don R., *Augusta, Georgia*
Gant, Norman F., *Dallas, Texas*
Garcia, Celso-Ramon, *Philadelphia, Pennsylvania*
Garfield, Robert E., *Hamilton, Ontario, Canada*
Garite, Thomas, *Orange, California*
Gates, Elana, *San Francisco, California*
Gath, Dennis, *Oxford, England*
Gazzaniga, Alan B., *Orange, California*
Gerbie, Albert, *Chicago, Illinois*
Gerbie, Melvin, *Chicago, Illinois*
Gerhardt, Kenneth, *Gainesville, Florida*
Gershenson, David, *Houston, Texas*
Gibbons, William E., *Houston, Texas*
Gibbs, Charles, *Denver, Colorado*
Gibbs, Ronald S., *Denver, Colorado*
Gibson, Mark, *Burlington, Vermont*
Gilbert, B.C., *York, England*
Gilbert, Raymond D., *Loma Linda, California*
Gilstrap, Larry C., III, *Dallas, Texas*
Gimovsky, Martin L., *Los Angeles, California*
Gleicher, Norblert, *Chicago, Illinois*
Gluck, Louis, *Orange, California*
Gluckman, P.D., *Auckland, New Zealand*
Gocke, Stephen E., *Palm Springs, California*
Golbus, Mitchell, *San Francisco, California*
Goldberg, James, *San Francisco, California*
Goldberg, Jeff, *Columbus, Ohio*
Golde, Steven H., *Burbank, California*
Goldenberg, Davis, *Newark, New Jersey*
Goldenberg, Robert, *Birmingham, Alabama*
Goldkrand, John, *Savannah, Georgia*
Goldstein, Donald P., *Boston, Massachusetts*
Goldstein, Philip, *Baltimore, Maryland*
Goldstein, Steven, *New York, New York*
Goldzieher, Joseph W., *Houston, Texas*
Golichowski, Alan, *Indianapolis, Indiana*
Gomel, Victor, *Vancouver, British Colubmia, Canada*
Gonik, Bernard, *Houston, Texas*
Goodlin, Robert, *Denver, Colorado*
Goodwin, Betsy, *Columbus, Ohio*
Granger, Gale A., *Irvine, California*
Grannum, Peter, *New Haven, Connecticut*
Gravett, Michael G., *Boise, Idaho*
Greene, John, *Lexington, Kentucky*
Greene, Michael, *Boston, Massachusetts*
Greenspoon, Jeffrey, *Los Angeles, California*
Greenwood, Frederick C., *Honolulu, Hawaii*
Greer, Benjamin E., *Seattle, Washington*
Greiss, Frank C. Jr., *Mooresville, North Carolina*
Grimes, David A., *Los Angeles, California*
Grimm, E.A., *Houston, Texas*
Grossman, John H., III., *Washington, D.C.*
Grundy, Howard, *Chicago, Illinois*
Guengerich, F. Peter, *Nashville, Tennessee*
Gupta, Sudhir, *Orange, California*
Gusdon, John P., *Jonesboro, Georgia*
Guzinski, Gay M., *Seattle, Washington*
Haas, Gil, *Oklahoma City, Oklahoma*
Hadley, Mack, *Tucson, Arizona*
Hadlock, Frank, *Houston, Texas*
Halberg, Franz, *Minneapolis, Minnesota*
Hale, Ralph, *Honolulu, Hawaii*
Hall, Douglas C., *Gainesville, Florida*
Hallman, Mikko, *Orange, California*
Halme, Jouko, *Chapel Hill, North Carolina*
Hammarback, S., *Umea, Sweden*
Hammond, Charles B., *Durham, North Carolina*
Hammond, Mary G., *Chapel Hill, North Carolina*
Haney, Arthur, *Durham, North Carolina*
Haning, Ray V., *Providence, Rhode Island*
Hankins, Gary, *Lackland, Texas*
Hans, Sydney, *Chicago, Illinois*
Hansen, Thomas N., *Houston, Texas*
Hanson, Frederick W., *Davis, California*
Harbert, Guy Jr., *Charlottesville, Virginia*
Harger, James H., *Pittsburgh, Pennsylvania*
Hart, Jayne L., *Fairfax, Virginia*
Haseltine, Florence, *Bethesda, Maryland*
Haspels, A., *Utrecht, Netherlands*
Hathaway, William E., *Denver, Colorado*
Hatjis, Christos George, *Columbus, Ohio*
Hauth, John C., *Birmingham, Alabama*
Hayashi, Robert H., *Ann Arbor, Michigan*
Hayashi, Terry T., *Pittsburgh, Pennsylvania*
Hazzard, William R., *Winston-Salem, North Carolina*
Hecht, Bryan, *Chicago, Illinois*
Hegge, Frederick, *Portland, Oregon*
Heinrichs, W. Leroy, *Stanford, California*
Henderson, Brian, *Los Angeles, California*
Henderson, Perry A., *Madison, Wisconsin*
Hendricks, Charles, *Chapel Hill, North Carolina*
Hendricks, Susan, *Seattle, Washington*
Hensleigh, Paul A., *Stanford, California*
Herbst, Arthur Lee, *Chicago, Illinois*
Herzog, David, *Boston, Massachusetts*
Hess, Allen D., *Baltimore, Maryland*
Hickok, Durlin E., *Seattle, Washington*
Hicks, Dorothy Jane, *Miami, Florida*
Hickson, Robert, *Chicago, Illinois*
Hidekazu, Hiraike, *Kyoto, Japan*
Hilgers, Robert, *Springfield, Illinois*
Hill, Edward, *San Francisco, California*
Hill, George, *Nashville, Tennessee*
Hill, Lyndon M., *Pittsburgh, Pennsylvania*
Hill, Washington C., *Omaha, Nebraska*
Hillier, Sharon, *Seattle, Washington*
Hobbins, John C., *New Haven, Connecticut*
Hobel, Calvin J., *Los Angeles, California*
Hodgen, Gary, *Norfolk, Virginia*
Hoffman, David I., *Chicago, Illinois*
Hofmann, Glen E., *New York, New York*
Hogge, W. Allen, *Baltimore, Maryland*
Hollander, Daniel, *Irvine, California*
Hollingsworth, Dorothy, *San Diego, California*
Holt, John, *Chicago, Illinois*
Homesley, Howard D., *Winston-Salem, North Carolina*
Hon, Edward, *Bradbury, California*
Honore, Louis, *Edmonton, Alberta, Canada*

Horger, Edgar O., III., *Charleston, South Carolina*
Hoskins, William John, *New York, New York*
Howard, Randy B., *Denver, Colorado*
Howie, Michael, *Columbus, Ohio*
Huddleston, John F., *Atlanta, Georgia*
Hughes, Claude, *Durham, North Carolina*
Hughes, David A., *London, England*
Hulka, Barbara, *Chapel Hill, North Carolina*
Hulka, Jaroslav, *Chapel Hill, North Carolina*
Hull, Magdalen, *Stoney Brook, New York*
Hunt, Joan, *Kansas City, Kansas*
Hurt, Glen, *Richmond, Virginia*
Huszar, Gabor B., *New Haven, Connecticut*
Hutchinson, Karen, *New Haven, Connecticut*
Hwang, Dan, *Baton Rouge, Louisiana*
Hytten, Frank E., *London, England*
Iams, Jay, *Columbus, Ohio*
Iams, Pat, *Columbus, Ohio*
Iannaccone, Philip, *Chicago, Illinois*
Ingardia, Charles, *Hartford, Connecticut*
Israel, Robert, *Los Angeles, California*
Jackson, David N., *Orange, California*
Jackson, Laird, *Philadelphia, Pennsylvania*
Jackson, Rebecca, *Columbus, Ohio*
Jaffe, Eric A., *New York, New York*
Jaffee, Robert B., *San Francisco, California*
James, L. Stanley, *New York, New York*
Jeyendran, Rajasingam, *Chicago, Illinois*
Johnson, Frances, *Columbus, Ohio*
Johnson, John W.C., *Gainesville, Florida*
Johnson, Paul, *Oxford, England*
Johnson, Spencer F., *Washington, D.C.*
Johnson, Timothy, *Baltimore, Maryland*
Jones, Colin T., *Oxford, England*
Jones, D. Yvonne, *Bethesda, Maryland*
Jones, Georgeanna, *Norfolk, Virginia*
Jones, Howard W., Jr., *Norfolk, Virginia*
Jones, Howard Wilber, III, *Nashville, Tennessee*
Jones, Malcolm D., *Orange, California*
Jones, Robert B., *Indianapolis, Indiana*
Josimovich, John B., *Brooklyn, New York*
Jouppila, Penttii, *Oulu, Finland*
Judd, Howard L., *Los Angeles, California*
Kahn-Dawood, Firyal S., *Houston, Texas*
Kaiser, Irwin, *Bronx, New York*
Kalra, Satya P., *Gainesville, Florida*
Kaminetzky, Harold, *Washington, D.C.*
Katz, David, *Orange, California*
Katz, Vern, *Chapel Hill, North Carolina*
Kaufman, Raymond H., *Houston, Texas*
Kauppila, Antti, *Oulu, Finland*
Kazer, Ralph R., *Chicago, Illinois*
Keegan, Kirk A., *Orange, California*
Keith, Louis, *Chicago, Illinois*
Kelly, John V., *Phoenix, Arizona*
Kennedy, Melanie, *Columbus, Ohio*
Keye, William R., *Salt Lake City, Utah*
Khoury, Muin, *Atlanta, Georgia*
Killam, Allen P., *Durham, North Carolina*
Kim, Moon H., *Columbus, Ohio*
Kinch, Robert, *Montreal, Quebec, Canada*
Kinsella, John, *Davis, California*
Kirschbaum, Thomas, *Los Angeles, California*
Kirz, Donna S., *Chicago, Illinois*
Kitchin, James D., III., *Charlottesville, Virginia*
Kitzmiller, John L., *San Francisco, California*
Klein, Luella, *Atlanta, Georgia*
Klerman, Lorraine, *New Haven, Connecticut*
Kletzky, Oscar, *Torrance, California*
Klopper, Arnold, *Aberdeen, Scotland*
Knapp, Robert C., *Boston, Massachusetts*
Kniss, Doug, *Columbus, Ohio*
Knopp, Robert H., *Seattle, Washington*
Knuppel, Robert A., *Tampa, Florida*
Kochenour, Neil K., *Salt Lake City, Utah*
Kofinas, George D., *Brooklyn, New York*
Kohler, Peter O., *Portland, Oregon*
Kohn, N.N., *Willingboro, New Jersey*
Kohorn, Ernest I., *New Haven, Connecticut*
Kola, Ismail, *Melbourne, Victoria, Australia*
Kretchmer, Norman, *San Francisco, California*
Kuhlman, Kathleen, *Philadelphia, Pennsylvania*
Kuhnert, Betty R., *Philadephia, Pennsylvania*
Kullander, Stig, *Malmo, Sweden*
Kurman, Robert John, *Baltimore, Maryland*
LaRosa, John C., *Washington, D.C.*
Lacey, Conley, *La Jolla, California*
Lafond, J., *Montreal, Quebec, Canada*
Lagrew, David C. *Laguna Hills, California*
Lamb, Emmet J., *Stanford, California*
Landon, Mark B., *Columbus, Ohio*
Langer, Oded, *San Antonio, Texas*
Lanzendorf, Susan E., *Chicago, Illinois*
Laragh, John, *New York, New York*
Laros, Russell K., *San Francisco, California*
Larsen, John, *Washington, D.C.*
Laube, Douglas W., *Iowa City, Iowa*
Lauersen, Niels, *New York, New York*
Lavin, Justin, *Akron, Ohio*
Lazarus, Gerald, *Philadelphia, Pennsylvania*
Leach, Charles, *New Britain, Connecticut*
Ledger, William J., *New York, New York*
Leduc, Line, *Houston, Texas*
Lee, Chi-Yu Gregory, *Vancouver, British Columbia, Canada*
Lee, N.C., *Atlanta, Georgia*
Leech, R.W., *Oklahoma City, Oklahoma*
Lenke, Roger, *Denver, Colorado*
Leppert, Phyllis, *New York, New York*
Leveno, Kenneth J., *Dallas, Texas*
Levey, Gerald S., *Pittsburgh, Pennsylvania*
Levinson, Carl, *San Francisco, California*
Lewandowski, George, *Columbus, Ohio*
Lewis, John, *New York, New York*
Lewis, Stephen, *Dallas, Texas*
Liggins, G.C., *Auckland, New Zealand*
Lindfors, Karen K., *Sacramento, California*
Lindheimer, Marshall, *Chicago, Illinois*
Lindsay, Michael K., *Atlanta, Georgia*
Lipshitz, Jeffrey, *Las Vegas, Nevada*

Little, Brian A., *Montreal, Quebec, Canada*
Liu, James H., *Cincinnati, Ohio*
Lobo, Rogerio A., *Los Angeles, California*
Lockwood, Charles, *New York, New York*
Longo, Lawrence D., *Loma Linda, California*
Loring, Theodore, *Eureka, California*
Louria, Donald, *Newark, New Jersey*
Low, James, *Kingston, Ontario, Canada*
Lowe, Nancy, *Columbus, Ohio*
Lowery, Curtis L., Jr., *Little Rock, Arkansas*
Lubbe, W.F., *Auckland, Australia*
Luciano, Anthony, *Farmington, Connecticut*
Luger, Anton, *Bethesda, Maryland*
Lurain, John R., *Chicago, Illinois*
Mabie, William, *Memphis, Tennessee*
MacCluer, Jean, *San Antonio, Texas*
MacDonald, Paul, *Dallas, Texas*
MacKay, H. Trent, *Sacramento, California*
Macri, James, *Carle Place, New York*
Magness, Ronald R., *Dallas, Texas*
Mahesh, Virendra, *Augusta, Georgia*
Main, Denise, *San Francisco, California*
Makowski, Edgar L., *Denver, Colorado*
Malinak, Lewis Russell, *Houston, Texas*
Malkasian, Goerge, *Rochester, Minnesota*
Mandelbrot, Laurent, *Paris, France*
Manetta, Alberto, *Orange, California*
Manning, Frank A., *Winnipeg, Manitoba, Canada*
March, Charles M., *Los Angeles, California*
Marchbanks, Polly, *Atlanta, Georgia*
Marrs, Richard P., *Los Angeles, California*
Marshall, Jean, *Providence, Rhode Island*
Marshall, John C., *Ann Arbor, Michigan*
Martin, Chester B., Jr., *Madison, Wisconsin*
Martin, James, *Jackson, Mississippi*
Marx, Gertie, *Bronx, New York*
Masterson, Byron J., *Gainesville, Florida*
Mastroianni, Luigi, *Philadelphia, Pennsylvania*
Mathur, Subbi, *Charleston, South Carolina*
Mattison, Donald R., *Pittsburgh, Pennsylvania*
Maulik, Dev, *Kansas City, Missouri*
Mazzaferri Ernest, *Columbus, Ohio*
McCoshen, John, *Winnipeg, Manitoba, Canada*
McCracken, John, *Shrewsbury, Massachusetts*
McDonald, John A., *Columbus, Ohio*
McDonough, Paul G., *Augusta, Georgia*
McGregor, James A., *Denver, Colorado*
McLachlan, Robert I., *Melbourne, Victoria, Australia*
McLaughlin, Margaret K., *Burlington, Vermont*
McLeod, Allan G.W., *Miami, Florida*
McMurray, Robert, *Chapel Hill, North Carolina*
McNellis, Donald, *Bethesda, Maryland*
McQuarrie, Howard G., *Salt Lake City, Utah*
McWilliams, Diana, *St. Louis, Missouri*
Mead, Philip, *Burlington, Vermont*
Medearis, Arnold L., *Rancho Palos Verdes, California*
Meis, Paul J., *Winston-Salem, North Carolina*
Meldrum, David R., *Rancho Palos Verdes, California*
Mendell, Jerry, *Columbus, Ohio*
Menge, Alan, *Ann Arbor, Michigan*
Mennuti, Michael T., *Philadelphia, Pennsylvania*
Mercer, Lane, *Chicago, Illinois*
Merkatz, Irwin R., *Bronx, New York*
Merrill, James A., *Seattle, Washington*
Meschia, Giacomo, *Denver, Colorado*
Mestman, Jorge H., *Los Angeles, California*
Metcalfe, James, *Portland, Oregon*
Metzger, Boyd E., *Chicago, Illinois*
Mikhail, George, *Sylmar, California*
Mikuta, John J., *Philadelphia, Pennsylvania*
Milewich, Leon, *Dallas, Texas*
Miller, David S., *Chicago, Illinois*
Miller, Frank C., *Little Rock, Arkansas*
Miller, John P., *San Bernardino, California*
Miller, Josephine B., *Chicago, Illinois*
Miller, Kurt, *Columbus, Ohio*
Miller, Richard K., *Rochester, New York*
Miller, Wayne, *Lincoln, Massachusetts*
Milunsky, Aubrey, *Boston, Massachusetts*
Minkoff, Howard, *Brooklyn, New York*
Mintz, Marshall, *Norfolk, Virginia*
Miodovnik, Menachem, *Cincinnati, Ohio*
Mishell, Daniel R., *Los Angeles, California*
Mitchell, Murray, *Salt Lake City, Utah*
Moawad, Atef H., *Chicago, Illinois*
Moghissi, Kamran S., *Detroit, Michigan*
Moise, Kenneth J., Jr., *Houston, Texas*
Molitor, John J., *Fullerton, California*
Monif, Gilles RG., *Omaha, Nebraska*
Moore, H. Christopher, *Orange, California*
Moore, Thomas R., *San Diego, California*
Morley, George, *Ann Arbor, Michigan*
Morrison, John C., *Jackson, Mississippi*
Morrow, C. Paul, *Los Angeles, California*
Mortel, Rodrique, *Hershey, Pennsylvania*
Morton, Mark, *Portland, Oregon*
Moscow, Jeffrey, *Bethesda, Maryland*
Moss. G.S., *Chicago, Illinois*
Motew, Martin N., *Chicago, Illinois*
Moutquin, Jean, *Quebec, Quebec, Canada*
Moyer, Dean L., *Los Angeles, California*
Mueller, Charles, *Columbus, Ohio*
Mueller-Heubach, Eberhard, *Winston-Salem, North Carolina*
Mullen, P.E., *Dunedin, New Zealand*
Munsick, Robert, *Indianapolis, Indiana*
Muram, David, *Memphis, Tennessee*
Murata, Yuji, *Orange, California*
Muse, Kenneth, *Lexington, Kentucky*
Myers, Stephen A., *Chicago, Illinois*
Naeye, Richard L., *Hershey, Pennsylvania*
Naftolin, Fred, *New Haven, Connecticut*
Nagamani, Manubai, *Galveston, Texas*
Nageotte, Michael, *Long Beach, California*
Nagey, David, *Baltimore, Maryland*
Nakamura, Robert M., *Los Angeles, California*

Nash, John, *Washington, D.C.*
Nathanielsz, Peter W., *Ithaca, New York*
Neff, John, *Columbus, Ohio*
Nelson, Donald Michael, *St. Louis, Missouri*
Nelson, George H., *Columbia, South Carolina*
Nelson, Karin B., *Bethesda, Maryland*
Nelson, Lewis W., *Winston-Salem, North Carolina*
Nelson, Sharon H., *Galveston, Texas*
Newman, Roger B., *Charleston, South Carolina*
Newton, Edward, *San Antonio, Texas*
Newton, Niles, *Chicago, Illinois*
Nichols, David, *Providence, Rhode Island*
Niebyl, Jennifer R., *Iowa City, Iowa*
Niswander, Kenneth R., *Sacramento, California*
Nochimson, David J., *North Granby, Connecticut*
Notelovitz, Morris, *Gainesville, Florida*
Novy, Miles, *Beaverton, Oregon*
Nowicki, Phillip, *Columbus, Ohio*
Nuwayhid, Bahij, *Los Angeles, California*
O'Brien, Timothy, *Little Rock, Arkansas*
O'Brien, William, *Tampa, Florida*
O'Shaughnessy, Richard W., *Columbus, Ohio*
O'Sullivan, Mary Jo, *Miami, Florida*
O'Toole, Robert, *Columbus, Ohio*
Oehninger, Sergio, *Arlington, Virginia*
Ogata, Edward, *Chicago, Illinois*
Ogburn, Paul, *Rochester, Minnesota*
Oi, Richard H., *Sacramento, California*
Olefsky, Jerrol, *La Jolla, California*
Olive, David L., *San Antonio, California*
Olson, david M., *London, Ontario, Canada*
Oparil, Suzanne, *Birmingham, Alabama*
Ory, Steven J., *Rochester, Minnesota*
Osteen, Kevin, *Nashville, Tennessee*
Ostergard, Donald R., *Long Beach, California*
Overstreet, James W., *Davis, California*
Pagon, Roberta, *Seattle, Washington*
Palomaki, Glenn, *Scarborough, Maine*
Paneth, Nigel, *East Lansing, Michigan*
Panigel, M., *Paris, France*
Papiernik, Emile, *Clamart, France*
Para, Michael, *Columbus, Ohio*
Parer, Julian T., *San Francisco, California*
Pariser, Steve, *Columbus, Ohio*
Parisi, Valerie M., *Houston, Texas*
Park, Robert C., *Washington, D.C.*
Parker, C. Richard Jr., *Birmingham, Alabama*
Parmley, Timothy H., *Little Rock, Arkansas*
Parsons, Anna, *Tampa, Florida*
Pastorek, Joseph G., *New Orleans, Louisiana*
Patrick, John E., *London, Ontario, Canada*
Patsner, Bruce, *Lakeland, Florida*
Patterson, Roy, *Chicago, Illinois*
Pattillo, Roland A., *Milwaukee, Wisconsin*
Pauerstein, Carl, *San Antonio, Texas*
Pauker, S.G., *Boston, Massachusetts*
Paul, Richard H., *Los Angeles, California*
Paulson, Richard, *Los Angeles, California*
Pedersen, Jan Fog, *Copenhagen, Denmark*
Pennell, Rebecca, G., *Doylestown, Pennsylvania*
Pent, David, *Phoenix, Arizona*
Peterson, Herbert B., *Atlanta, Georgia*
Peterson, Robert G., *Ottawa, Ontario, Canada*
Petrie, Roy H., *St. Louis, Missouri*
Phelan, Jeffrey P., *West Covina, California*
Phillippe, Mark, *Chicago, Illinois*
Pilkington, Joseph W., *St. Petersburg, Florida*
Pitkin, Roy M., *Los Angeles, California*
Pittaway, Donald Edward, *Clemmons, North Carolina*
Piver, Steven, *Buffalo, New York*
Platt, Lawerence D., *Los Angeles, California*
Plowman, P.N., *London, England*
Plows, Charles, *Anaheim, California*
Podczaski, Edward S., *Hershey, Pennsylvania*
Podratz, Karl C., *Rochester, Minnesota*
Polak, J.F., *Boston, Massachusetts*
Polan, Mary L., *Stanford, California*
Poland, Marilyn, *Detroit, Michigan*
Pope, J. Terrell, *Macon, Georgia*
Porreco, Richard P., *Denver, Colorado*
Porto, Manuel, *Orange, California*
Powell, Dwight, *Columbus, Ohio*
Power, Gordon G., *Loma Linda, California*
Powers, David, *Orange, California*
Press, M.F., *Chicago, Illinois*
Prior, Jerilynn, *Vancouver, British Columbia, Canada*
Queenan, John T., *Washington, D.C.*
Quigley, M.E. Ted, *San Diego, California*
Quilligan, Edward J., *Orange, California*
Quirk, J. Gerald, *Little Rock, Arkansas*
Rabe, Thomas, *Heidelberg, West Germany*
Randall, N.J., *London, England*
Rankin, John H.G., *Madison, Wisconsin*
Ranney, Brooks, *Yankton, South Dakota*
Rapkin, Andrea J., *Los Angeles, California*
Rasmussen, Dennis D., *La Jolla, California*
Ratnoff, Oscar, *Cleveland, Ohio*
Ravnikar, Veronica A., *Boston, Massachusetts*
Rayburn, William F., *Omaha, Nebraska*
Read, John A., *Tacoma, Washington*
Rebar, Robert W., *Cincinnati, Ohio*
Redman, Christopher, *Oxford, England*
Redpath, Leslie J., *Irvine, California*
Redwine, Fay O., *Richmond, Virginia*
Reece, E. Albert, *New Haven, Connecticut*
Reed, Kathryn, *Tucson, Arizona*
Reid, Robert L., *Kingston, Ontario, Canada*
Reiss, Rosemary E., *Columbus, Ohio*
Resnik, Robert, *La Jolla, California*
Reynolds, W. Ann, *Long Beach, California*
Richardson, Bryan, *London, Ontario, Canada*
Richart, Ralph M., *New York, New York*
Riddick, Daniel, *Burlington, Vermont*
Rieder, Michael, *London, Ontario, Canada*
Rigatto, Henrique, *Winnipeg, Manitoba, Canada*
Rightmire, Daniel A., *Springfield, Illinois*
Robboy, Stanley, *Newark, New Jersey*
Roberts, A.B., *New Haven, Connecticut*
Roberts, Daniel, *Wichita, Kansas*
Roberts, James M., *San Francisco, California*

Roberts, William S., *Tampa, Florida*
Robertson, D.M., *Melbourne, Victoria, Australia*
Rock, John A., *Baltimore, Maryland*
Rodis, John, *Farmington, Connecticut*
Rogers,Robert, *Indianapolis, Indiana*
Rojas, Francisco J., *Orange, California*
Rolla, Giovanni, *Torino, Italy*
Romero, Roberto, *New Haven, Connecticut*
Romney, Seymour, *Bronx, New York*
Rose, James C., *Winston-Salem, North Carolina*
Rosen, Gregory F., *Orange, California*
Rosen, Mortimer, *New York, New York*
Rosenberg, R.D., *Boston, Massachusetts*
Rosenblum, Norman, *Philadelphia, Pennsylvania*
Rosene, Karen, *Providence, Rhode Island*
Rosenfeld, Charles, *Dallas, Texas*
Rosenfeld, Ron, *Stanford, California*
Rosenfield, Allan, *New York, New York*
Rosenfield, Robert L., *Chicago, Illinois*
Rosenthal, Miriam, *Cleveland, Ohio*
Ross, Michael G., *Torrance, California*
Rossavik, Ivar K., *Oklahoma City, Oklahoma*
Rovner, David R., *East Lansing, Michigan*
Roy, Subir, *Los Angeles, California*
Rubin, Robert, *Torrance, California*
Rudolph, Abe M., *San Francisco, California*
Rulin, Marvin C., *Pittsburgh, Pennsylvania*
Rurak, Dan W., *Vancouver, British Columbia, Canada*
Russell, Keith, *Los Angeles, California*
Rust, Lyman A., *Hanford, California*
Ryan, George, *Memphis, Tennessee*
Ryan, Kenneth, *Boston, Massachusetts*
Sabbagha, Rudy E., *Chicago, Illinois*
Sachs, Larry A., *Columbus, Ohio*
Sacks, David, *Los Alamitos, California*
Sadovsky, E., *Jerusalem, Israel*
Sahn, David, *San Diego, California*
Said, Sami I., *Chicago, Illinois*
Saks, Bonnie Rae, *Tampa, Florida*
Salafia, CArolyn, *Farmington, Connecticut*
Saller, Devereux, *Providence, Rhode Island*
Saltzman, Daniel., *Boston, Massachusetts*
Samaan, Naguib A., *Houston, Texas*
Samuels, Philip, *Philadephia, Pennsylvania*
Sanchez-Ramos, Luis, *Jacksonville, Florida*
Sander, C. Maureen, *East Lansing, Michigan*
Sanfilippo, Joseph, *Louisville, Kentucky*
Sarno, Albert P., *Honolulu, Hawaii*
Sarto, Gloria E., *Albuquerque, New Mexico*
Sauer, Mark V., *Los Angeles, California*
Sbarra, A., *Boston, Massachusetts*
Scanlon, John W., *Washington, D.C.*
Schaefer, Ernest J., *Boston, Massachusetts*
Schaefer, George, *La Mesa, California*
Schiff, Isaac, *Boston, Massachusetts*
Schifrin, Barry, *Tarzana, California*
Schink, Julian, *Chicago, Illinois*
Schneider, Jack M., *Sacramento, California*
Schneider, V., *Ulm, West Germany*
Schreiber, James, *Chicago, Illinois*
Schulman, Harold, *Mineola, New York*
Schwartz, Peter E., *New Haven, Connecticut*
Schwartz, Robert, *Providence, Rhode Island*
Schwartz, Ruth, *Rochester, New York*
Schwarz, Richard, *New York, New York*
Scioscia, Angela, *San Diego, California*
Scommegna, Antonio, *Chicago, Illinois*
Scott, James R., *Salt Lake, City, Utah*
Sealey, Jean, *New York, New York*
Sedlis, Alexander, *Brooklyn, New York*
Seeds, John W., *Chapel Hill, North Carolina*
Segars, James, *Bethesda, Maryland*
Seibel, Machelle M., *Chestnut Hill, Massachusetts*
Seppala, Markku, *Helskini, Finland*
Sever, John L., *Washington, D.C.*
Shah, Dinesh M., *Nashville, Tennessee*
Shangold, Mona M., *Philadelphia, Pennsylvania*
Shanklin, Douglas, *Memphis, Tennessee*
Shapiro, Sander S., *Madison, Wisconsin*
Shaw, Kathryn J., *Los Angeles, California*
Shaw, Thomas, *Chicago, Illinois*
Sheld, Harrison H., *Las Vegas, Nevada*
Shenker, Lewis, *Tucson, Arizona*
Sher, Geoffrey, *San Francisco, California*
Sherins, Richard J., *Fairfax, Virginia*
Sherline, Donald M., *Augusta, Georgia*
Sherwood, O.D., *Urbana-Champaign, Illinois*
Shingleton, Hugh M., *Birmingham, Alabama*
Shmoys, Susan, *Stony Brook, New York*
Shnider, Sol M., *San Francisco, California*
Shoupe, Donna, *Los Angeles, California*
Shulman, Lee, *Memphis, Tennessee*
Shulman, Sidney, *New York, New York*
Shwartz, Michael, *Boston, Massachusetts*
Sibai, Baha, *Memphis, Tennessee*
Siddiqi, Tariq, *Cincinnati, Ohio*
Siiteri, Pentti, *San Francisco, California*
Siler-Khodr, Theresa M., *San Antonio, Texas*
Simpson, Joe Leigh, *Memphis, Tennessee*
Sjoberg, Nils Otto, *Malmo, Sweden*
Sleep, Jennifer, *Hampshire, England*
Smith, Carl H., *St. Louis, Missouri*
Smith, Julian P., *Maywood, Illinois*
Smith, Lloyd H., *Sacramento, California*
Smith, Michael R., *Seattle, Washington*
Smith, Moyra, *Irvine, California*
Smith, Nina, *Columbus, Ohio*
Smotkin, David, *Salt Lake City, Utah*
Sobel, Jack D., *Philadelphia, Pennsylvania*
Socol, Michael L, *Chicago, Illinois*
Soderstrom, Richard M., *Seattle, Washington*
Sokol, Robert J., *Detroit, Michigan*
Sommer, Annemarie, *Columbus, Ohio*
Sonek, Jiri, *Augusta, Georgia*
Sparks, John, *Denver, Colorado*
Spellacy, William N., *Tampa, Florida*
Sperelakis, Nicholas, *Cincinnati, Ohio*
Speroff, Leon, *Portland, Oregon*
Spitz, B., *Leuven, Belgium*
Spruance, S.L., *Salt Lake City, Utah*

Stanczyk, Frank Z., *Los Angeles, California*
Stanley, Richard, *Bronx, New York*
Stehman, Frederick B., *Indianapolis, Indiana*
Stenchever, Morton, *Seattle, Washington*
Stevens, Vernon, *Columbus, Ohio*
Stiller, Robert, *Bridgeport, Connecticut*
Stillman, Robert J., *Washington, D.C.*
Stirrat, Gordon M., *Bristol, England*
Stjernquist, Martin, *Malmo, Sweden*
Stolley, Paul D., *Philadelphia, Pennsylvania*
Stone, Sergio C., *Orange, California*
Strauss, Jerome F., III, *Philadelphia, Pennsylvania*
Stubblefield, Phillip G., *Portland, Maine*
Stubbs, Thomas, *Charleston, South Carolina*
Stull, J.T., *Dallas, Texas*
Stumpf, Walter, *Chapel Hill, North Carolina*
Sullivan, Jay M., *Memphis, Tennessee*
Surwit, Earl, *Tucson, Arizona*
Sutton, Gregory, *Indianapolis, Indiana*
Sweet, Craig R., *Augusta, Georgia*
Sweet, Richard L., *San Francisco, California*
Sykes, G.S., *Hornsby, Australia*
Symonds, E. Malcolm, *Nottingham, England*
Szulman, Aron, *Pittsburgh, Pennsylvania*
Tabsh, Khalil M.A., *Los Angeles, California*
Talamantes, Frank, *Santa Cruz, California*
Talbert, Luther M., *Chapel Hill, North Carolina*
Tamura, Ralph K., *Chicago, Illinois*
Tatemoto, K., *Fukuoka, Japan*
Tatum, Howard James, *Atlanta, Georgia*
Taylor, J. Bruce, *Charlotte, North Carolina*
Taylor, Robert N., *San Francisco, California*
Taylor, Stewart, *Denver, Colorado*
Temple, R. Henry, *Wilmington, North Carolina*
Teng, Nelson, *Stanford, California*
Thiede, Henry A., *Rochester, New York*
Thomason, Jessica Love, *Milwaukee, Wisconsin*
Thompson, John D., *Atlanta, Georgia*
Thompson, Joyce Beebe, *Philadelphia, Pennsylvania*
Thompson, William B., *Orange, California*
Thornburg, Kent L., *Portland, Oregon*
Thrupp, Lauri D., *Orange, California*
Thurnau, Gary, *Oklahoma City, Oklahoma*
Timor-Tritsch, Ilan E., *New York, New York*
Tobias, John, *Orange, California*
Toledo, Andre, *Atlanta, Georgia*
Tomich, Paul, *Maywood, Illinois*
Toner, James P., *Norfolk, Virginia*
Towers, Craig V., *Long Beach, California*
Townsend, Duane E., *Sacramento, California*
Triplett, Douglas A., *Muncie, Indiana*
Trudinger, Brian J., *Westmead, Australia*
Tseng, Linda, *Stony Brook, New York*
Tsibris, John C., *Tampa, Florida*
Twiggs, Leo B., *Minneapolis, Minnesota*
Tyler, Carl, *Decatur, Georgia*
Tyson, John E.A., *London, Ontario, Canada*
Tyson, Jon E., *Dallas, Texas*
Ueland, Kent, *Lahaina, Hawaii*
Underwood, Paul B., *Charlottesville, Virginia*
Urnes, Paul D., *Chicago, Illinois*
Utian, Wulf, *Cleveland, Ohio*
Valenzuela, Guillermo J., *Loma Linda, California*
Valle, Rafael F., *Chicago, Illinois*
Van Assche, Andre, *Leuven, Belgium*
Van Den Noort, Stanley, *Irvine, California*
Van Dorsten, James Peter, *Richmond, Virginia*
Van Geijn, Herman, *Amsterdam, Holland*
Veille, Jean-Claude, *Cleveland, Ohio*
Vener, Kirt, *Bethesda, Maryland*
Vermesh, Michael, *Tarzana, California*
Villar, Jose, *Geneva, Switzerland*
Vintzileos, Anthony, *Farmington, Connecticut*
Voda, A.M., *Salt Lake City, Utah*
Volpe, Joseph, *St. Louis, Missouri*
Vontver, Louis, *Seattle, Washington*
Waffarn, Feizal, *Orange, California*
Wallach, Edward E., *Baltimore, Maryland*
Wallenburg, Henk C.S., *Rotterdam, Netherlands*
Walsh, Scott, *Richmond, Virginia*
Wapner, Ronald J., *Philadelphia, Pennsylvania*
Warren, James C., *St. Louis, Missouri*
Warren, John R., *Chicago, Illinois*
Warsof, Steven L., *Chicago, Illinois*
Wasmuth, John J., *Irvine, California*
Webb, Gilbert, *San Francisco, California*
Weinberg, Brice, *Durham, North Carolina*
Weiner, Carl P., *Iowa City, Iowa*
Weiner, Stuart, *Philadelphia, Pennsylvania*
Weingold, Allan, *Washington, D.C.*
Weinstein, Louis, *Tucson, Arizona*
Weiss, Gerson, *Newark, New Jersey*
Weiss, Noel, *Seattle, Washington*
Welander, Charles, *Atlanta, Georgia*
Wells, K.B., *Los Angeles, California*
Wendel, Earl F., *Chicago, Illinois*
Wentz, Anne, *Chicago, Illinois*
Wettstein, Felix, *Los Angeles, California*
Wharton, James Taylor, *Houston, Texas*
Wheeler, A.S., *Tuscaloosa, Alabama*
Wheeless, Clifford, *Baltimore, Maryland*
Wheller, John, *Columbus, Ohio*
White, James R., *La Jolla, California*
Whitley, Richard, *Birmingham, Alabama*
Whitman, Gail G., *Simsbury, Connecticut*
Whitsett, Jeffrey, *Cincinnati, Ohio*
Whittle, Martin J., *Glasgow, Scotland*
Wiesmeir, Edward, *Encino, California*
Wilbanks, George D., *Chicago, Illinois*
Wiley, Lynne, *Davis, California*
Wilken-Jensen, Charlotte, *Glostrup, Denmark*
Wilkin, Jonathan, *Columbus, Ohio*
Williams, Ronald L., *Santa Barbara, California*
Williamson, H. Oliver, *Charleston, South Carolina*
Wiser, Winifred L., *Jackson, Mississippi*
Witkin, Steven, *New York, New York*
Witter, Frank, *Baltimore, Maryland*
Woessner, Frederick, *Miami, Florida*
Wolfe, C.D.A., *London, England*
Wolfson, Robert N., *Colorado Springs, Colorado*

Wolverton, Stephen, *Columbus, Ohio*
Wood, Charles E., *Gainesville, Florida*
Woodruff, J. Donald, *Baltimore, Maryland*
Woods, James R., *Rochester, New York*
Work, Bruce A., Jr., *Minneapolis, Minnesota*
Wurtman, Judith, *Cambridge, Massachusetts*
Wurtman, Richard, *Cambridge, Massachusetts*
Wynn, Ralph M., *Pontiac, Michigan*
Yee, Bill, *Orange, California*
Yeh, Sze-Ya, *Allentown, Pennsylvania*
Yen, Samuel S.C., *La Jolla, California*
Yeomans, Edward, *Lackland, Texas*
Yonekura, Margaret Lynn, *Torrance, California*
Young, Bruce K., *New York, New York*
Young, Peter, *Indianapolis, Indiana*
Young, Ronald, *Houston, Texas*
Youngs, David D., *Cleveland, Ohio*
Zackman, Richard D., *Madison, Wisconsin*
Zacur, Howard, *Baltimore, Maryland*
Zatuchni, Gerald I., *Chicago, Illinois*
Zebelman, Arthur, *Seattle, Washington*
Zuspan, Frederick P., *Columbus, Ohio*

Is intrauterine transfusion associated with diminished fetal growth?

**Gregory O. Utter, MD,[a] Michael L. Socol, MD,[a] Sharon L. Dooley, MD,[a]
Scott N. MacGregor, DO,[a] and Dietra D. Millard, MD[b]**
Chicago, Illinois

Studies in animal models and human pregnancies suggest that severe fetal anemia and/or replacement of fetal with adult blood result in decreased pH, increased base deficit, and hyperlactacidemia. Similar changes have been noted in growth-retarded, nonanemic fetuses, and we therefore hypothesized that isoimmunized fetuses requiring intrauterine transfusions might have diminished growth. We longitudinally studied growth patterns in 17 isoimmunized fetuses by noting biparietal diameter and head and abdominal circumference measurements at each transfusion. The distributions of these measurements above and below the 25th, 50th, and 75th percentiles derived from our general obstetric population were compared at the initial transfusion and the last ultrasonogram performed before delivery. Birth weights also were noted and their distribution around the 25th, 50th, and 75th percentiles was compared to the expected distribution. For each ultrasonographic parameter, the distribution of measurements at the last ultrasonogram before delivery was not significantly different from the distribution at the initial ultrasonogram. The birth weight distribution also was not significantly different than the expected distribution. Thus we were unable to demonstrate slowing of fetal growth in our severely isoimmunized pregnancies. (Am J Obstet Gynecol 1990;163:1781-4.)

Key words: Fetal anemia, intrauterine transfusion, diminished fetal growth

Hyperlactacidemia and increased base deficit have been detected in umbilical cord blood on nonanemic, growth-retarded fetuses.[1, 2] The proposed mechanism of altered fetal growth in these pregnancies is uteroplacental insufficiency, which is associated with abnormal transplacental delivery of metabolic substrates such as amino acids and glucose.[3-5] The hyperlactacidemia and increased base deficit, however, suggest that fetal growth retardation may also be secondary to reduced transplacental oxygen transport with subsequent fetal hypoxemia. High-altitude studies of fetal growth are consistent with the premise that perturbations in fetal oxygen delivery may play a role in the pathophysiology of diminished fetal growth.[6, 7]

Fetal anemia also can result in abnormal fetal oxygen delivery as a result of reduced oxygen-carrying capacity.[8] In the sheep model biochemical parameters consistent with fetal hypoxia and acidosis are noted only with severe fetal anemia,[8, 9] suggesting that the fetus can compensate for moderate anemia with an increase in oxygen extraction and thereby maintain normal oxygen consumption. On the other hand, after replacement of fetal blood with adult blood, even moderate anemia results in metabolic acidosis.[9] Comparable to the animal model, in isoimmunized human pregnancies only severely anemic fetuses show a decrease in oxygen delivery and concomitant metabolic acidosis.[10-12] However, when compared with nontransfused fetuses with similar hemoglobin levels, in those fetuses transfused with adult blood, hyperlactacidemia and increased base deficit develop, suggestive of relative hypoxia.[13]

As there are qualitative similarities in the acid-base status of nonanemic, growth-retarded fetuses and anemic, transfused isoimmunized fetuses, we hypothesized that in severely isoimmunized pregnancies the profound fetal anemia and/or replacement of fetal blood volume with adult red blood cells might be associated with diminished fetal growth.

Material and methods

Seventeen nonhydropic fetuses affected by Rh or Kell isoimmunization and requiring intrauterine intravascular transfusion were studied. Ultrasonographic biparietal diameter and head and abdominal circumfer-

From the Sections of Maternal-Fetal Medicine[a] and Neonatalogy,[b] Departments of Obstetrics and Gynecology and Pediatrics, Northwestern University Medical School, Northwestern Memorial Hospital, and the Evanston Hospital.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Gregory O. Utter, MD, St. Clair Professional Building, Suite 313, 22151 Moross Road, Detroit, MI 48236.
6/6/24772

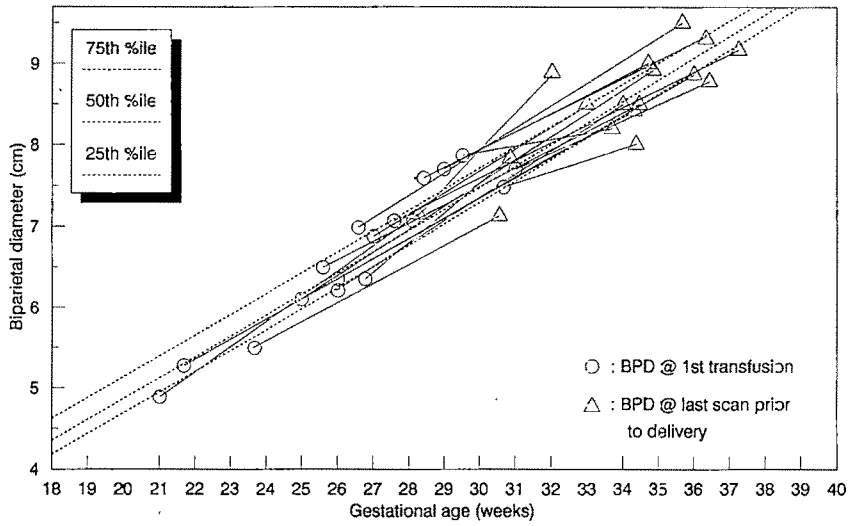


Fig. 1. Biparietal diameter distribution at the last ultrasonogram before delivery was not different than the distribution at the initial transfusion.

Table I. Obstetric and transfusion data

Patient No.	*Gestational age at first tranfusion (wk)*	*Pretransfusion hematocrit (%)*	*Total No. of transfusions*	*Kleihauer-Betke (% fetal cells).*	*Gestational age at delivery (wk)*	*Birth weight (gm)*	*Birth weight percentile*
1	25	20.9	4	4.6	36	2270	10th-25th
2	30	20.5	2	0	34	2680	75th-90th
3	22	24.4	4	0	35	2630	50th-75th
4	29	12.1	2	1.9	35	2925	50th-75th
5	32	20.8	1	40	36	2120	<10th
6	27	16.6	3	0	35	2865	50th-75th
7	27	15.8	3	0.1	33	2020	50th-75th
8	30	16.8	2	1.5	35	3220	75th-90th
9	26	25.3	4	0.1	36	2760	25th-50th
10	26	14.0	3	0	32	1700	25th-50th
11	28	10.8	4	0	37	2980	50th-75th
12	22	15.1	4	0	29	1390	25th-50th
13	29	10.1	3	0	36	2985	75th-90th
14	29	23.5	2	0	35	1630	<10th
15	26	28.0	4	1	36	2630	25th-50th
16	26	26.0	4	1	34	2611	75th-90th
17	24	24.4	4	1	31	1505	25th-50th

ence measurements were noted at the time of each transfusion. The distributions of these measurements above and below the 25th, 50th, and 75th percentiles derived from our general obstetric population[14] were compared at the initial transfusion and the last ultrasonogram performed before delivery. Birth weight percentile was determined for each infant from Brenner's growth curve[15] and the birth weight distribution above and below the 25th, 50th, and 75th percentiles for gestational age was then compared with the expected distribution.

Measurement distributions around the 25th, 50th, and 75th percentiles were compared by means of χ^2 analysis, with Fisher's exact test where appropriate. All p values <0.05 were considered statistically significant.

Results

Obstetric and transfusion data for each patient are displayed in Table I. The mean gestational age at initial transfusion was 27.0 ± 2.8 weeks, and the mean hematocrit at the initial transfusion was 19.1% ± 5.6%. The mean number of transfusions per fetus was 3 ± 1, and the mean interval from the initial transfusion to delivery was 7.3 ± 2.5 weeks. The Kleihauer-Betke acid elution test was performed on umbilical cord blood at the time of the last transfusion or at birth and showed that in 16 of 17 fetuses <5% of red blood cells showed positive stains for fetal hemoglobin.

Figs. 1, 2, and 3 display biparietal diameter, head circumference, and abdominal circumference measurements from the ultrasonogram performed at the

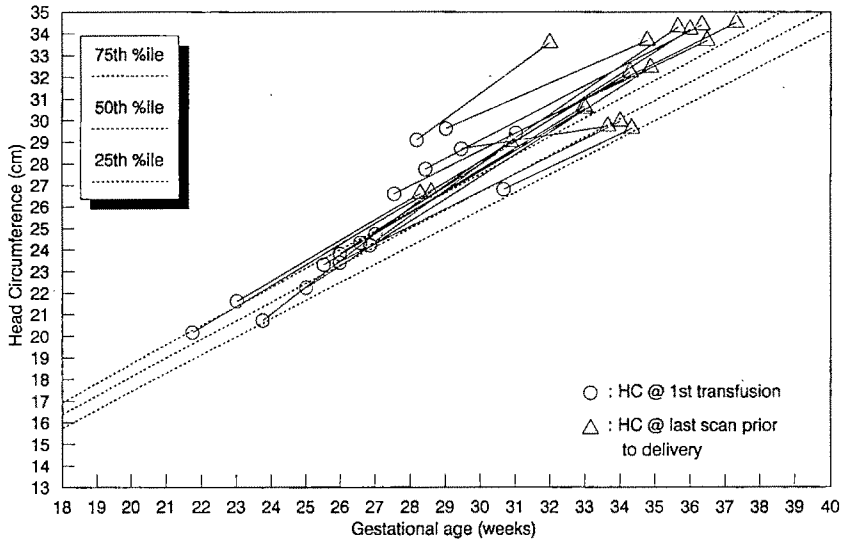


Fig. 2. Head circumference distribution at the last ultrasonogram before delivery was not different than the distribution at the initial transfusion.

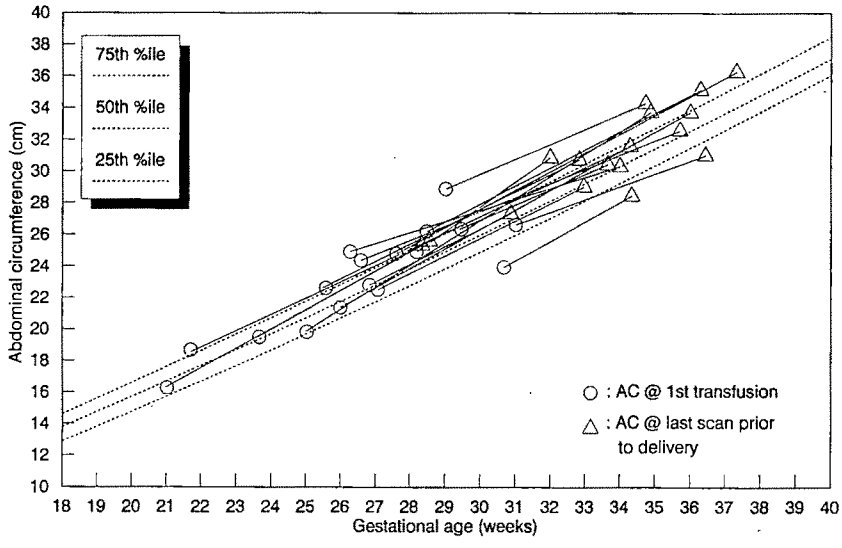


Fig. 3. Abdominal circumference distribution at the last ultrasonogram before delivery was not different than the distribution at the initial transfusion.

initial transfusion and from the last ultrasonogram before delivery, superimposed on the 25th, 50th, and 75th percentile curves derived from our general obstetric population. The distributions of the biparietal diameter, head circumference, and abdominal circumference measurements at the last ultrasonogram before delivery were not significantly different from the distributions at the initial ultrasonogram, nor were the distributions of the biparietal diameter or abdominal circumference at either ultrasonogram significantly different from that of our general obstetric population. The head circumference distribution at the final ultrasonogram revealed significantly more measurements above the 75th percentile ($\chi^2 = 5.85$, $p < 0.05$). The birth weight distribution was not significantly different from the expected distribution.

Comment

The biochemical consequences of fetal anemia and intrauterine transfusions with adult blood have been examined in animal models and in human pregnancies via umbilical vessel blood sampling. These studies consistently suggest that severe fetal anemia and/or replacement of fetal blood with adult blood result in relative hypoxia, with decreasing pH, increased base deficit, and hyperlactacidemia.[8-13] The clinical significance

of these findings is uncertain, but it has been postulated that these changes make the fetus more susceptible to hypoxia during acute stress, i.e., during labor. To our knowledge, however, no one has longitudinally studied the potential for more long-term effects of anemia and intrauterine transfusion on fetal development. We postulated that the relative hypoxia and acidemia attributable to anemia or intrauterine replacement of fetal blood with adult blood might result in diminished fetal growth.

After studying growth patterns in our 17 anemic, transfused fetuses, we were unable to demonstrate slowing of fetal growth despite nearly total replacement of fetal blood with adult red blood cells. Slowing of fetal growth after the onset and treatment of anemia would have resulted in more measurements at the final ultrasonogram being distributed below each percentile when compared with the ultrasonographic measurements at the initial transfusion. However, we found that the distribution of standard morphometric measurements around the 25th, 50th, and 75th percentiles remained unchanged over the interval from the initial transfusion to delivery and was comparable to the distribution of the general obstetric population. Also, the distribution of birth weight percentiles was not different than expected.

The fetuses in this study were severely anemic at the time of the initial transfusion, and subsequent transfusions were performed when fetal hematocrit was estimated to be 20% to 25%. Further, the interval from the first transfusion to birth was sufficiently long to allow for and enable the detection of altered intrauterine growth. Consequently, our observations suggest that alterations in oxygenation parameters that may be significant at the biochemical level are not manifested clinically by diminished fetal growth.

REFERENCES

1. Nicolaides KH, Economides DL, Soothill PW. Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol 1989;161:996-1001.
2. Soothill PW, Nicolaides KH, Campbell S. Prenatal asphyxia, hyperlacticaemia, hypoglycemia, and erthyroblastosis in growth retarded fetuses. Br Med J 1987; 294:1051-3.
3. Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Evans MI. Plasma amino acids in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol 1989; 161:1219-27.
4. Economides DL, Proudler A, Nicolaides KH. Plasma insulin in appropriate- and small-for-gestational-age fetuses. Am J Obstet Gynecol 1989;160:1091-4.
5. Nicolini U, Hubinont C, Santolaya J, Fisk NM, Coe AM, Rodeck CH. Maternal-fetal glucose gradient in normal pregnancies and in pregnancies complicated by alloimmunization and fetal growth retardation. Am J Obstet Gynecol 1989;161:924-7.
6. Kruger H, Arias-Stella J. The placenta and the newborn infant at high altitudes. Am J Obstet Gynecol 1970; 106:586-91.
7. Lichty JA, Ting RY, Bruns PD, Dyar E. Studies of babies born at high altitude. Am J Dis Child 1957;93:666-9.
8. Edelstone DI, Caine ME, Fumia FD. Relationship of fetal oxygen consumption and acid base balance to fetal hematocrit. Am J Obstet Gynecol 1985;151:844-51.
9. Edelstone DI, Darby MJ, Bass K, Miller K. Effects of reductions in hemoglobin-oxygen affinity and hematocrit level on oxygen consumption and acid-base state in fetal lambs. Am J Obstet Gynecol 1989;160:820-8.
10. Soothill PW, Nicolaides KH, Rodeck CH. Effect of anaemia on fetal acid-base status. Br J Obstet Gynaecol 1987;94:880-3.
11. Soothill PW, Nicolaides KH, Rodeck CH, Clewell WH, Lingridge J. Relationship of fetal hemoglobin and oxygen content to lactate concentration in Rh isoimmunized pregnancies. Obstet Gynecol 1987;69:268-71.
12. Westgren M, Selbing A, Stangenberg M, Philips R. Acid-base status in fetal heart blood in erythroblastotic fetuses: a study with special reference to the effect of transfusions with adult blood. Am J Obstet Gynecol 1989;160: 1134-8.
13. Soothill PW, Nicolaides KH, Rodeck CH, Bellingham AJ. The effect of replacing fetal hemoglobin with adult hemoglobin on blood gas and acid-base parameters in human fetuses. Am J Obstet Gynecol 1988;158:66-9.
14. Sabbagha RE. Intrauterine growth retardation. In: Sabbagha RE, ed. Diagnostic ultrasound applied to obstetrics and gynecology. Philadelphia: JB Lippincott, 1987:112-31.
15. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. Am J Obstet Gynecol 1976;126:555-64.

Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus

Dru E. Carlson, MD, Lawrence D. Platt, MD, Arnold L. Medearis, MD, and Janet Horenstein, MD

Los Angeles, California

To delineate any possible prognostic indicators, we reviewed the ultrasonographic and nonmorphometric findings in 19 antenatally diagnosed cases of nonimmune hydrops fetalis in which it was chosen to continue the pregnancy. Thirteen fetuses died and six survived. Of all parameters examined, including associated anomalies, abnormal karyotype, location of serous fluid, anemia, and possible cause of nonimmune hydrops fetalis, the most sensitive prognostic indicator was the real-time-directed M-mode echocardiographic measurement of the biventricular outer dimension in diastole. All fetuses with biventricular outer dimensions >95% died, whereas all but one with normal biventricular outer dimensions had resolution of nonimmune hydrops fetalis and survived. This was highly significant ($p < 0.001$) with the predictive value of a normal biventricular outer dimension being 86% and the predictive value of an enlarged biventricular outer dimension being 100%. (AM J OBSTET GYNECOL 1990;163:1785-7.)

Key words: Nonimmune hydrops fetalis, M-mode echocardiography, prenatal diagnosis

Nonimmune hydrops fetalis is defined as an excessive amount of total body water that results in extracellular accumulation of fluid in tissues and serous cavities. It occurs specifically without evidence of antibodies to red blood cell antigens. The ultrasonographic diagnosis of hydrops fetalis can be made by identifying two or more fluid-filled spaces within the affected fetus.[1] For example, there can be pleural effusions, pericardial effusions, ascites, and skin edema. There are more than 80 causes of this condition, and the incidence is approximately 1 in 3000 deliveries.[2, 3] The perinatal death rate is 40% to 90%.[2-4] However, some fetuses do survive. Until now there has not been a specific ultrasonographic finding that is a good predictor of perinatal prognosis. Therefore the purpose of this study was to retrospectively determine the best prognostic indicator among routine prenatal ultrasonographic measurements and observations obtained in pregnancies with nonimmune hydrops fetalis.

Methods

We reviewed all cases of prenatally diagnosed nonimmune hydrops fetalis seen in our Genetics Clinic at Women's Hospital of Los Angeles County–University of Southern California Medical Center from July 1986 to June 1989. Inclusion into the study required a living fetus at the time of diagnosis and the mother electing to continue the pregnancy after diagnosis was made. All patients were offered percutaneous umbilical blood sampling and/or amniocentesis to attempt to find a cause of the nonimmune hydrops fetalis. The usual noncardiac ultrasonographic measurements were obtained, including the biparietal diameter, circumferences of the head, chest, and abdomen, femur length, placental thickness, and amniotic fluid index. In addition, we routinely obtain real-time cardiac images and real-time-directed M-mode echocardiography on every abnormal fetus. The M-mode cursor was placed perpendicular to the interventricular septum at the level of the mitral and tricuspid valves. In this way the M-mode displays the pericardium, ventricular walls, ventricular chambers and valves, and septum. These landmarks were then measured in diastole. We specifically recorded the biventricular outer dimension (BVOD) and the biventricular inner dimension (BVID). The BVOD is measured epicardium to epicardium. The BVID is defined by the inner wall of one chamber to the inner wall of the other chamber. Thus the BVID measures the size of the chambers and septum but the walls and epicardium are excluded (Fig. 1). The M-mode echocardiographic measurements were plotted against the biparietal diameter to correlate with gestational age. Normal values are from the 5% to the 95% confidence intervals. Abnormally enlarged values are greater than the 95% confidence interval. These parameters were previously delineated at our institution by DeVore et al.[5-8]

The noncardiac morphometric measurements, all

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine.

Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, March 21-24, 1990, St. Louis, Missouri.

Reprint requests: Dr. Dru E. Carlson, Department of Obstetrics and Gynecology, University of Southern California School of Medicine, 1240 North Mission Road, Room 5K40, Los Angeles, CA 90033.

6/6/24762

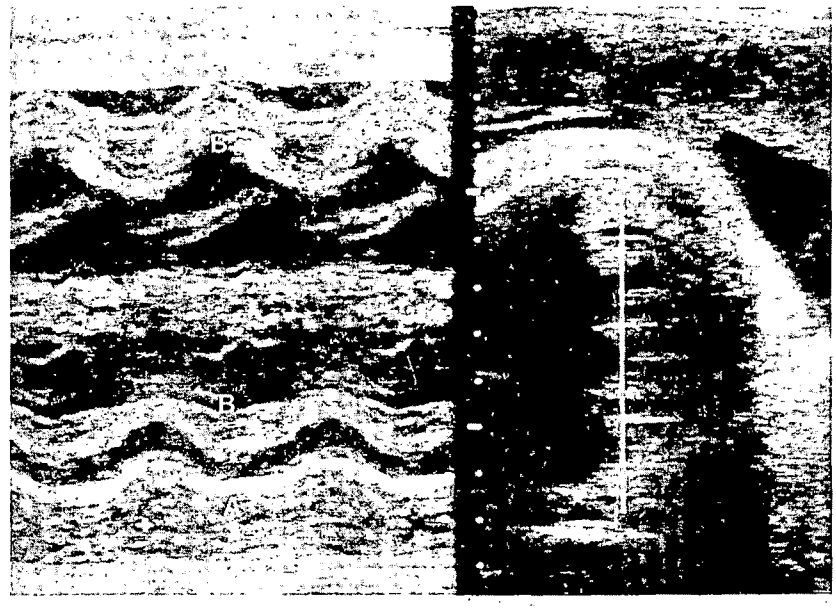


Fig. 1. On the right is the real-time image of the fetal heart showing the M-mode cursor in a perpendicular transection of the four-chamber view. On the left is the M-mode image. *A* to *A*, BVOD in diastole; *B* to *B*, BVID in diastole.

Table I. Nonmorphometric prognostic indicators

Parameter	*Survived**	*Died**
Pleural effusion	3/6 (50%)	10/13 (77%)
Pericardial effusion	3/6 (50%)	9/13 (69%)
Ascites	6/6 (100%)	13/13 (100%)
Skin edema	3/6 (50%)	10/13 (77%)
Associated anomaly	1/6 (16%)	3/13 (15%)
Abnormal karyotype	0/6 (0%)	2/13 (15%)

*p = NS for all parameters.

Table II. M-Mode echocardiographic prognostic indicators

BVOD	*Survived*	*Died*
>95%	0*	12*
≤95%	6*	1*

*$p < 0.001$.

nonmorphometric observations, and M-mode echocardiographic evaluations were recorded at the initial diagnosis of nonimmune hydrops fetalis.

Results

Nineteen consecutive patients met the inclusion criteria. They ranged from 22.5 to 35 weeks' gestation at the time of diagnosis. Of the 19, 6 survived and 13 died—6 in the antepartum period and 7 in the neonatal period. The causes of the 13 deaths included osteogenesis imperfecta type 2, cytomegalovirus, *Pseudomonas*, parvovirus, Noonan syndrome, and Turner syndrome. Both of the latter two cases had cystic hygroma without evidence of coarctation of the aorta. There was arthrogryposis of undetermined cause in two cases, and causes of nonimmune hydrops fetalis were unknown in four cases.

Of the six survivors, only one had a known etiology of the nonimmune hydrops fetalis—a unilateral cystic adenomatoid malformation of the lung. No other cause of nonimmune hydrops fetalis could be determined by prenatal or postnatal investigations, which included viral cultures, karyotype, electrophoresis, and parvovirus probe. Table I shows the nonmorphometric parameters observed. Statistical analysis of these nonmorphometric parameters by Fisher's exact test did not differentiate the survivors from those who died.

Two fetuses who survived had invasive procedures during the antenatal period. One had a thoracentesis and one had a paracentesis; neither had a reaccumulation of fluid in those spaces after the procedure. Four fetuses who died also had invasive procedures. One had a thoracentesis and three had paracentesis. However, all had prompt reaccumulation of fluid in that space.

When we examined our M-mode echocardiographic parameters (Table II), we found that all fetuses with enlarged BVODs died and all fetuses but one with normal BVODs lived. This was highly significant by Fisher's exact test at the level of $p < 0.001$. The sensitivity of the BVOD is 92%. The specificity is 100%. The predictive value of an enlarged BVOD is 100% and the predictive value of a normal BVOD is 86%.

The one fetus that died with a normal BVOD had

an abnormal karyotype (mos 46,XY/47,XY,+20) with severe arthrogryposis and polyhydramnios. The mosaicism was diagnosed by percutaneous umbilical blood sampling at 22.5 weeks, and this karyotype was confirmed at autopsy after the fetus died in utero at 24.5 weeks.

All fetuses with normal BVODs also had normal BVIDs. However, there was variation of the BVID in the 12 fetuses with enlarged BVODs who died. Eight of these 12 had normal BVIDs. Six of those eight had thickened ventricular walls and overall cardiac hypertrophy accounting for the enlarged BVOD. These fetuses included four with neonatal death (osteogenesis imperfecta type 2, parvovirus with endocardial fibroelastosis, and two of unknown causes) and two who died antenatally (Noonan syndrome and Turner syndrome).

The remaining two of eight fetuses with enlarged BVODs but normal BVIDs had massive pericardial effusions that resulted in apparent cardiac tamponade. One of those fetuses had arthrogryposis of unknown cause and died at <4 hours of life; the other died antenatally with nonimmune hydrops fetalis of unknown causes.

Four fetuses had enlarged BVIDs and BVODs, and they all had thin ventricular walls and dilated ventricular chambers. These included two that died neonatally (one with a *Pseudomonas* septicemia and one of unknown cause). There were two antepartum deaths (one with α-thalassemia major and one with cytomegalovirus infection).

Anemia as measured by percutaneous umbilical blood sampling or at delivery was not a major cause of the nonimmune hydrops fetalis in any of these cases. The hematocrits ranged from 32% to 55%.

Autopsy findings and/or neonatal echocardiography confirmed our M-mode echocardiographic impressions in all cases.

The six fetuses who survived presently range in age from 8 months to 2.5 years and according to their parents are progressing as well as their siblings with no apparent sequelae from the nonimmune hydrops fetalis. However, none have had formal developmental testing and school performances are pending.

Comment

Of the parameters measured in this study of prenatally diagnosed nonimmune hydrops fetalis, the BVOD of the fetal heart was the best predictor of perinatal outcome; 12 of 12 fetuses with enlarged BVODs died and 6 of 7 fetuses with normal BVODs lived.

It is interesting that several other authors have found that supraventricular tachycardia is an important cause of nonimmune hydrops fetalis.[9, 10] In this study we found no fetuses with supraventricular tachycardia as the cause of nonimmune hydrops fetalis. We believe that this may be a function of our referral pattern within an indigent population.

Also one previous study reported an association between the area and amount of accumulation of fluids and the level of fetal anemia.[11] We found no evidence of significant anemia in any of the fetuses we studied, therefore it was not a good predictor of outcome.

In summary, we found that we could not predict the outcome of prenatally diagnosed fetuses with nonimmune hydrops fetalis by usual ultrasonography and/or nonmorphometric parameters. However, an enlarged BVOD as measured by M-mode echocardiography was an excellent prognosticator of fetal death. This enlarged BVOD may reflect different places on the spectrum of cardiac failure—an overall hypertrophy of the fetal heart, a large pericardial effusion, or dilated ventricular chambers with thin walls. A normal BVOD predicted all survivors of nonimmune hydrops fetalis but one. We hypothesize that the normal BVOD measurement seen in the surviving fetuses may be indicative of a less severe or compensated cardiac failure that is commonly associated with this entity.

REFERENCES

1. Platt LD, DeVore GR. In utero diagnosis of hydrops fetalis: ultrasound methods. Clin Perinatol 1982;9:627-36.
2. Hutchison AA, Drew JH, Yu VYH, et al. Nonimmunological hydrops fetalis: a review of 61 cases. Obstet Gynecol 1982;59:347-52.
3. Machin GA. Hydrops revisited; literature review of 1,414 cases published in the 1980s. Am J Med Genet 1989;34: 366-90.
4. Im SS, Rizos N, Joutsi P, et al. Nonimmunological hydrops fetalis. Am J Obstet Gynecol 1984;148:566-9.
5. DeVore GD, Horenstein J, Platt LD. Fetal echocardiography. Am J Obstet Gynecol 1986;155:1066-71.
6. DeVore GR, Donnerstein RL, Kleinman CS, Platt LD, Hobbins JC. Fetal echocardiography. I. Normal anatomy as determined by real-time–directed M-mode ultrasound. Am J Obstet Gynecol 1982;144:249-59.
7. DeVore GR, Donnerstein RL, Kleinman CS, Platt LD, Hobbins JC. Fetal echocardiography. II. The diagnosis and singificance of a pericardial effusion in the fetus using real-time–directed M-mode ultrasound. Am J Obstet Gynecol 1982;144:693-9.
8. DeVore GR, Siassi B, Platt LD. Fetal echocardiography. IV. M-Mode assessment of ventricular size and contractility during the second and third trimesters of pregnancy in the normal fetus. Am J Obstet Gynecol 1984;150: 981-8.
9. Kleinman CS, Donnerstein RL, DeVore GR, et al. Fetal echocardiography for evaluation of in utero congestive heart failure. N Engl J Med 1982;306:568-75.
10. Silverman NH, Kleinman CS, Rudolph AM, et al. Fetal atrioventricular valve insufficiency associated with nonimmune hydrops: a two-dimensional echocardiographic and pulsed Doppler ultrasound study. Circulation 1985; 72:825-32.
11. Maidman JE, Yeager C, Anderson V, et al. Prenatal diagnosis and management of nonimmunological hydrops fetalis. Obstet Gynecol 1980;56:571-6.

Sources of variability in umbilical artery systolic/diastolic ratios: Implications of the Poiseuille equation

Jeffrey W. Wright, MD, and Louis E. Ridgway, MD
San Antonio, Texas

The Poiseuille equation of fluid flow suggests that umbilical cord length and cord blood viscosity may affect resistance to flow and thus affect the systolic/diastolic ratio. In this prospective study of 40 uncomplicated term pregnancies, we sought to define the relationship of umbilical cord length and cord blood hematocrit level (as an index of viscosity) to the umbilical artery systolic/diastolic ratio. To focus on these factors, we minimized known sources of systolic/diastolic ratio variability and controlled for fetal heart rate. Linear regression revealed that fetal heart rate contributed 18% of the systolic/diastolic ratio variability. Conversely, umbilical cord length and cord blood hematocrit level were not related to umbilical artery systolic/diastolic ratio. We conclude that normal variations in cord blood hematocrit level and umbilical cord length do not significantly affect systolic/diastolic ratio. (Am J Obstet Gynecol 1990;163:1788-91.)

Key words: Doppler ultrasonography, velocimetry, vascular resistance

Doppler velocimetric measurement of umbilical artery systolic/diastolic (S/D) ratio is thought to be a measure of downstream placental resistance to blood flow. It would seem that such measurements should be very useful in the detection of patients with uteroplacental insufficiency.[1-3] However, it has proved difficult to clearly distinguish between the normal and abnormal in clinical practice.[4-7] This difficulty may be a result of variability in the measured S/D ratios that is unrelated to placental resistance. Many extraneous factors including fetal heart rate (FHR),[7-10] gestational age,[7, 11] site of insonation,[7] operator,[7] and type of Doppler device have been shown to affect the measured S/D ratio.

The Poiseuille equation delineates the factors that affect fluid flow (flow = $\Delta P \pi r^4 / 8nl$; where ΔP = pressure gradient, r = vessel radius, n = viscosity, and l = vessel length). It suggests that blood viscosity and umbilical cord length may affect umbilical artery vascular resistance. Thus these factors may affect the measured S/D ratio. The relationship of umbilical cord length and S/D ratio has not been studied. Previous studies[12, 13] have suggested that viscosity or hematocrit level may affect S/D ratio. However, the inclusion of patients with uteroplacental insufficiency and severe fetal anemia may have biased these results.

The goal of this prospective study was to define the relationship of umbilical artery S/D ratio to the umbilical cord length and cord blood hematocrit level (as an index of viscosity), while either minimizing or controlling for known sources of S/D ratio variability.

From the Department of Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Jeffrey W. Wright, MD, Department of Obstetrics and Gynecology, UTHSCSA, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836.
6/6/24729

Material and methods

In this prospective study, 40 patients first seen in active labor with intact membranes were examined. Active labor was defined as regular uterine contractions with cervical dilatation of 4 to 7 cm. Patients receiving epidural anesthetic agents or analgesic drugs were excluded. Potential sources of variance in S/D ratios were minimized by: (1) selection of only normal pregnancies at 38 to 41 weeks' gestation as determined by last menstrual period and confirmed by ultrasonography; (2) use of a single examiner (J. W.) and a single Doppler device (Angioscan III, Ultrasonix, Inc., North Yonkers, N.Y.); (3) exclusion of patients with any condition associated with uteroplacental insufficiency, fetal anemia, or fetal polycythemia. FHR was measured for inclusion as an independent variable to control for its effect on S/D ratio.

Continuous-wave Doppler with a 4 mHz transducer was used to obtain all S/D ratios. Patients were examined in the recumbent position with 15° to 30° of lateral uterine displacement. Readings were taken between contractions in the absence of fetal breathing or fetal body movement. Absence of fetal movement was confirmed by a combination of real-time ultrasonography, palpation, maternal perception, and the absence of characteristic changes in the Doppler waveforms. The Doppler probe was positioned on the abdomen to obtain the best quality umbilical artery signals, as recognized by the characteristic waveform and umbilical vein flow in the opposite direction. Peak systolic and lowest diastolic frequency shifts were measured with electronic calipers; S/D ratios were then calculated with five wave-

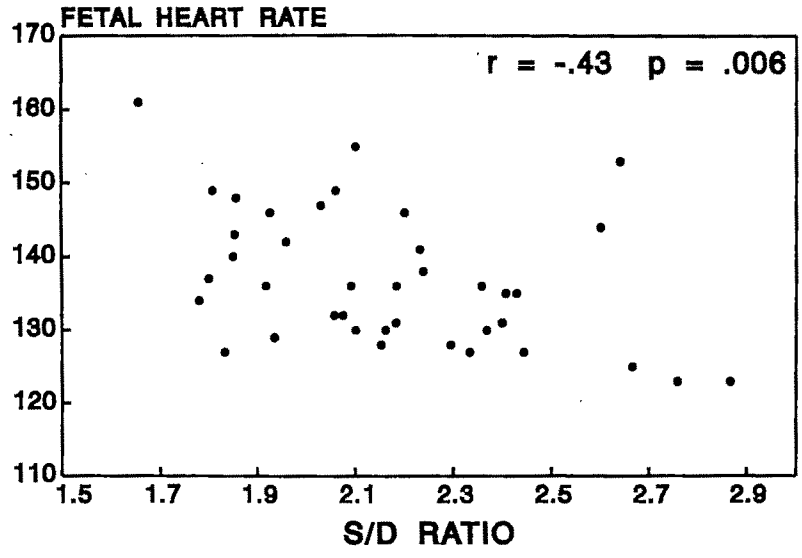


Fig. 1. Scatter plot of S/D ratio vs FHR (S/D = 4.0 − 0.013 · FHR).

forms averaged to obtain each reading. FHR was calculated by measuring the distance between waveform peaks with electronic calipers.

Umbilical cord blood was aspirated from the umbilical vein immediately after delivery and spun hematocrit obtained. Umbilical cord length was measured from fetal insertion to placental insertion with a paper tape measure.

Because S/D ratio is known to be non-normally distributed, we examined the skewness and kurtosis of the S/D ratio data and performed the Shapiro-Wilk's test for normality. We found that in this selected study population, the S/D ratios obtained were normally distributed. Thus parametric statistics were used. The three independent variables, cord length, hematocrit value, and FHR, were individually plotted against S/D ratio to assure that no nonlinear relationships existed. No nonlinear relationships were found. Individual Pearson's correlation coefficients were obtained. Multiple linear regression with all possible subsets was performed to establish the relationship between the independent variables and S/D ratio. An $\alpha < 0.05$ was used to determine significance. A biomedical data processing statistical package was used for the analysis.

Results

A total of 40 patients with a mean age of 23.8 ± 5.8 years and mean parity of 1.5 ± 1.5 were examined. All patients had normal fetal outcomes as defined by 5-minute Apgar scores >7, and normal neonatal physical examination. Table I summarizes the dependent and independent variables measured. The ranges of these variables indicate a reasonable breadth of values in our sample.

Scatter plots of S/D ratio versus FHR, cord blood hematocrit level, and umbilical cord length are displayed in Figs. 1, 2, and 3. No nonlinear relationship were found. FHR had the strongest correlation with S/D ratio ($r = -0.43$, $p = 0.006$). As expected, S/D ratio decreased as FHR increased. There was no correlation between cord blood hematocrit value and S/D ratio (Fig. 2). As seen on inspection of Fig. 3, there appears to be a slight negative correlation between S/D ratio and cord length. However, this is not statistically significant.

Table I. Summary of data obtained

	Mean ± SD	*Low*	*High*
S/D ratio	2.17 ± 0.29	1.66	2.87
Gestational age (wk)	39.6 ± 1.1	38	41
FHR (beats/min)	137.0 ± 9.2	123	161
Cord length (cm)	58.7 ± 12.9	40	95
Cord hematocrit value (%)	49.3 ± 6.2	40	67

Multiple linear regression was performed with all possible subsets. As predicted by the correlation coefficients, FHR was the only significant contributor to S/D ratio variability. Cord length and hematocrit level remained nonsignificant even after controlling for FHR with this regression. The equation obtained was S/D = 4.0 − 0.0134 × FHR ($r = 0.43$) ($p = 0.01$). The calculated r^2 of 0.18 indicates that changes in FHR account for 18% of the variability in the S/D ratio data.

To ensure that changes in S/D ratio associated with gestational age were not confounding our results, we tested the correlation of S/D ratio versus gestational age. There was no significant correlation with S/D ratio over this short span of gestational age (Spearman's rank correlation coefficient, $r = 0.002$). Further, the multi-

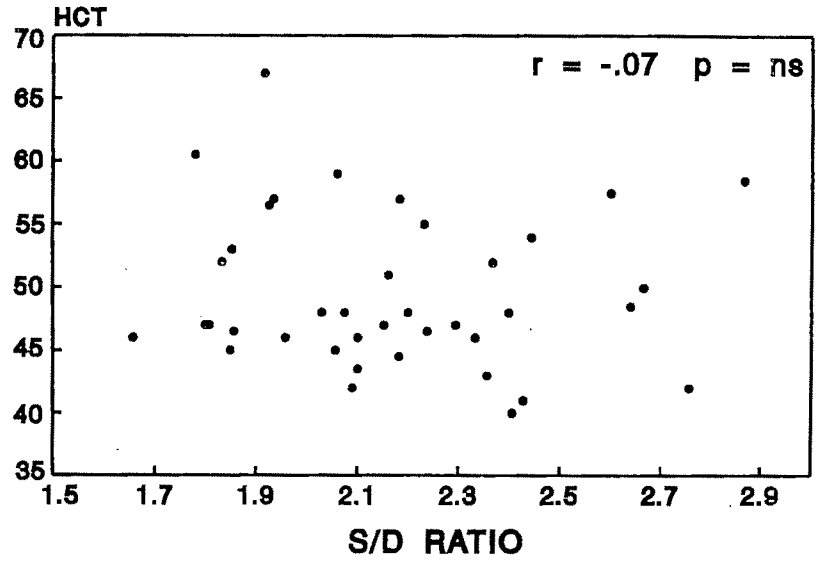


Fig. 2. Scatter plot of S/D ratio vs cord blood hematocrit value.

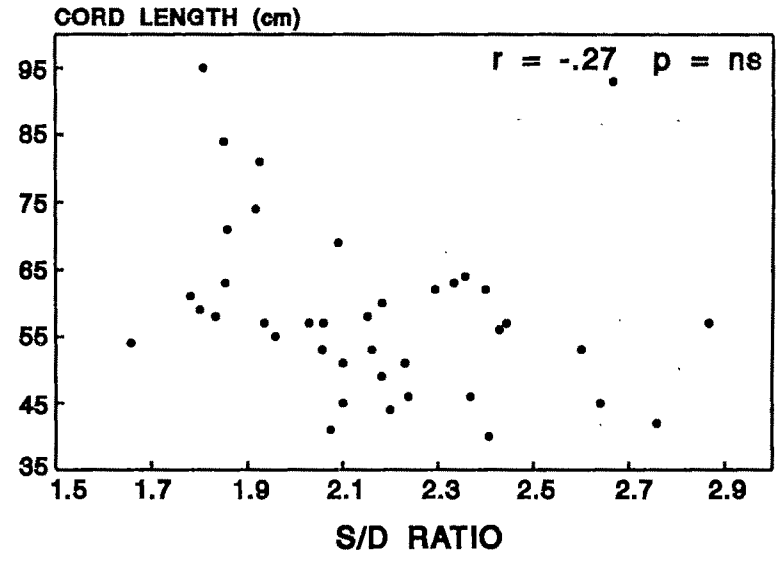


Fig. 3. Scatter plot of S/D ratio vs umbilical cord length.

ple linear regression was redone with the addition of gestational age as an independent variable. The results were unchanged.

Comment

After minimizing all other known sources of variability, we found that FHR was significantly related to S/D ratio in term pregnancies. In our study group, FHR accounted for 18% of the variability seen in the S/D ratios. We then controlled for the effect of FHR with multiple regression and were unable to show a significant relationship between S/D ratio and umbilical cord hematocrit level or umbilical cord length.

Our study confirms the findings of several previous authors[7-10] who reported that S/D ratio decreases as FHR increases. This is predictable, as slower heart rates allow the end-diastolic velocity to fall to a greater extent, resulting in a higher S/D ratio. The strength of this correlation was similar to that reported by Maulik[7] in a multivariate analysis.

In our study population, variations in hematocrit level had no relation to variation in S/D ratio. Giles and Trudinger[12] examined the relationship of S/D ratio to umbilical cord blood hematocrit level and viscosity. They found a significantly higher hematocrit level in a group of patients with elevated S/D ratios. This group also had complications of pregnancy associated with placental insufficiency. Thus both the elevated S/D ra-

tios and the higher hematocrit levels might have been related to placental insufficiency. Another explanation might be that hematocrit level affects S/D ratio only when the hematocrit level is very high. Such high hematocrit values are sometimes seen with intrauterine hypoxemia. However, the range of hematocrit levels we encountered was similar to that of Giles and Trudinger.[12]

Rightmire and colleagues[13] studied the effect of hematocrit level on umbilical artery velocimetry in a group of isoimmunized pregnancies. They found an inverse relationship between fetal hematocrit level and blood velocity in the descending aorta. The relationship between hematocrit value and Pourcelot's index (PI = (S − D)/S) was more complex, with the most severely anemic fetuses having paradoxically high measurements. These authors believed that a lower hematocrit value causes less viscosity and less resistance to flow. The paradoxically high resistance of the severely anemic fetuses was thought to be related to capillary damage and plugging. It is not appropriate to compare Rightmire's results[13] to the current study as the ranges of hematocrits values are different (6% to 38% vs 40% to 67%). Nevertheless, our data suggest that variations in hematocrit level within the normal range do not affect S/D ratio.

The slight inverse relationship of cord length and S/D ratio is contrary to what the Poiseuille equation predicts. We recognize that S/D ratios with continuous wave Doppler are measured at a variety of sites along the umbilical cord. It is possible that we may have found cord length to be a significant contributor to S/D ratio variability had we measured consistently at the fetal insertion with a duplex Doppler system. However, we chose continuous wave Doppler because these devices are more commonly available to the clinician.

A β error is the final possible explanation for our inability to show a correlation between S/D ratio and either cord length or cord hematocrit value. Power analysis with our sample size revealed an 85% chance of detecting a correlation similar to that found between S/D ratio and FHR.

Umbilical artery velocimetry is a promising tool for evaluation of the fetus. However, its current role as a clinical test is controversial. It is logical to think that placental insufficiency states would lead to elevated placental resistance and elevated S/D ratios. However, both false-negative and false-positive findings are common. The crux of the problem may lie in determining what is normal and what is abnormal. This study and those we have cited show that many factors contribute to the variability in the measurements obtained. We agree with Maulik[7] that controlling for known sources of variability may be the next step in the clinical application of Doppler velocimetry. Our study shows that normal variations in cord blood hematocrit level and umbilical cord length do not significantly contribute to this variability.

REFERENCES

1. Schulman H, Fleischer A, Stern W, Farmakides G, Jagani N, Blattner P. Umbilical velocity wave ratios in human pregnancy. AM J OBSTET GYNECOL 1984;148:985-90.
2. Bracero LA, Beneck D, Kirshenbaum N, Peiffer M, Stalter P, Schulman H. Doppler velocimetry and placental disease. AM J OBSTET GYNECOL 1989;161:388-93.
3. Trudinger BJ, Giles WB, Cook CM. Uteroplacental blood flow velocity-time waveforms in normal and complicated pregnancy. BR J OBSTET GYNAECOL 1985;92:39-45.
4. Divon MY, Girz BA, Lieblich R, Langer O. Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow. AM J OBSTET GYNECOL 1989; 161:1523-7.
5. Berkowitz GS, Chitkara U, Rosenberg J, et al. Sonographic estimation of fetal weight and Doppler analysis of umbilical artery velocimetry in the prediction of intrauterine growth retardation: a prospective study. AM J OBSTET GYNECOL 1988;158:1149-53.
6. Sijmons EA, Reuwer PJHM, Van Beek E, Bruinse HW. The validity of screening for small-for-gestational-age and low-weight-for-length infants by Doppler ultrasound. BR J OBSTET GYNAECOL 1989;96:557-61.
7. Maulik D, Yarlagadda AP, Youngblood JP, Willoughby L. Components of variability of umbilical arterial doppler velocimetry—a prospective analysis. AM J OBSTET GYNECOL 1989;160:1406-12.
8. Mires G, Dempster J, Patel NB, Crawford JW. The effect of fetal heart rate on umbilical artery flow velocity waveforms. BR J OBSTET GYNAECOL 1987;94:665-9.
9. Fairlie FM. The relation between fetal heart rate and Doppler flow velocity waveform A/B ratio. BR J OBSTET GYNAECOL 1988;95:312-4.
10. Brar HS, Medearis AL, Platt LD. Relationship of systolic/diastolic ratios from umbilical velocimetry to fetal heart rate. AM J OBSTET GYNECOL 1989;160:188-91.
11. Pearce JM, Campbell S, Cohen-Overbeek T, Hackett G, Herandez J, Royston JP. References ranges and sources of variation for indices of pulsed Doppler flow velocity waveforms from the uteroplacental and fetal circulation. BR J OBSTET GYNAECOL 1988;95:248-56.
12. Giles WB, Trudinger BJ. Umbilical cord whole blood viscosity and the umbilical artery flow velocity time waveforms: a correlation. BR J OBSTET GYNAECOL 1986; 93:466-70.
13. Rightmire DA, Nicolaides KH, Rodeck CH, Campbell S. Fetal blood velocities in Rh isoimmunization: relationship to gestational age and to fetal hematocrit. OBSTET GYNECOL 1986;68:233-6.

Variation and correlation in human fetal umbilical Doppler velocities with fetal breathing: Evidence of the cardiac-placental connection

Julia H. Indik, PhD, and Kathryn L. Reed, MD
Tucson, Arizona

Doppler velocity waveforms in the human fetal umbilical vein and artery were analyzed during episodes of fetal breathing. Heart rate, systolic and diastolic velocities were measured from the umbilical artery waveform. Diastolic velocity varied the most with a mean (± SD) coefficient of variation of 16.0% ± 5.0%. The coefficient of variation of systolic velocity was 7.8% ± 2.4% and of heart rate was 5.0% ± 1.8%. We also found that umbilical arterial flow was related to umbilical venous flow, implying an interdependence between fetal cardiovascular blood flow and placental blood flow. During breathing, venous flow varies because of changes in intrathoracic pressure in the fetus. This variation in umbilical venous velocity may affect the umbilical arterial diastolic velocity through alterations in placental filling, and may affect the umbilical arterial systolic velocity through alterations in ventricular filling, which by the Frank-Starling mechanism changes stroke volume. The interdependency of umbilical venous and umbilical arterial blood flow velocities must be considered in the interpretation of the significance of umbilical artery Doppler velocity measurements. (Am J Obstet Gynecol 1990;163:1792-6.)

Key words: Human fetal cardiovascular hemodynamics, Doppler ultrasonography, umbilical artery, umbilical vein, fetal breathing

Studies of human umbilical arterial Doppler blood flow velocity waveforms have shown that relative flow velocities during diastole increase with advancing gestation in normal fetuses.[1, 2] Because umbilical arterial velocities vary with fetal breathing movements, investigators have cautioned against measurement of waveforms obtained during fetal breathing.[3] Variations in umbilical venous blood flow velocities with fetal breathing have also been reported,[3-5] and, in fact, have been proposed as a means by which to recognize the presence of fetal breathing.

Disproportionately low diastolic blood flow velocities in the umbilical artery have been associated with intrauterine growth retardation.[1-3, 6] Investigators have attributed the low diastolic blood flow velocities to increased resistance in the organ perfused by umbilical arterial blood—the placenta.

In reviewing umbilical venous and arterial waveforms obtained during fetal breathing, we noted that certain patterns of variations occurred in these waveforms and hypothesized that these patterns could be quantitated. In addition, we hypothesized that these changes would not only confirm previous reports that the Frank-Starling mechanism was operative in the fetal heart,[7] but would further show that umbilical arterial waveforms vary in association with changes in umbilical venous velocities. These findings of interdependency between umbilical venous blood flow velocities and umbilical arterial blood flow velocities introduce the importance of the fetal cardiovascular function, in addition to placental function, in the determination of the umbilical arterial Doppler velocity waveform.

From the Department of Obstetrics and Gynecology, Arizona Health Sciences Center.
Funded in part by a grant from the American Heart Association, Arizona Affiliate.
Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Kathryn L. Reed, MD, Department of Obstetrics and Gynecology, Arizona Health Sciences Center, 1501 N. Campbell Ave., Tucson, AZ 85724.
6/6/24726

Methods

Ultrasonographic studies were performed in fetuses at risk of anomalies (positive family history, possible teratogen exposure) or growth abnormalities (size-date inconsistency). Two-dimensional ultrasonographic studies of fetal anatomy, size, and amniotic fluid volume were performed with 3.5 and 5.0 MHz transducers. The presence of fetal breathing was established by two-dimensional ultrasonography before and after umbilical Doppler recordings were obtained.

Umbilical venous and arterial Doppler velocities were obtained simultaneously during episodes of fetal breathing. Doppler studies were performed with a 3.5 MHz transducer. Approval for the study was obtained from the Human Subjects Committee, and informed consent was obtained from each participating mother.

Heart rate and umbilical arterial systolic and diastolic velocities were measured from strip chart recordings. The relative contribution of variation in heart rate, systolic and diastolic velocities to overall variation in the umbilical arterial waveform, was examined.

Umbilical arterial systolic and diastolic velocities and heart rate were measured with a digitizing board, and a mean value and SD were calculated for each variable in each fetus. A coefficient of variation ([SD/mean] × 100) was then determined for each variable in each fetus. For the three variables of umbilical arterial systolic velocity, umbilical arterial diastolic velocity, and heart rate, the coefficients of variation were averaged for the group of fetuses. In addition, to determine whether increases in variation of one variable were associated with increases in variation of another variable, coefficients of variation of heart rates of individual fetuses were compared with coefficients of variation of umbilical arterial diastolic velocities of individual fetuses; similarly, heart rate variations were compared with umbilical arterial systolic velocity variations, and umbilical arterial diastolic velocity variations were compared with umbilical arterial systolic velocity variations. For each fetus, a minimum of nine cardiac cycles was measured.

The Student t test was used to compare the coefficients of variation of umbilical arterial systolic and diastolic velocities with heart rate, with significance at $p < 0.05$. Linear regression analysis was used to correlate coefficients of variation for each variable in each fetus, again with significance at $p < 0.05$.

To determine whether any correlation was present between changes in umbilical arterial and venous flow, we selected fetuses in which velocities from both artery and vein were simultaneously observed for at least one breathing cycle. Typically, breathing cycles were at least twice as long as cardiac cycles; shorter breathing cycles were not analyzed. We further restricted our analysis to areas where the venous flow varied by at least two pixels (3.0 mm) in the tracings. A two-pixel limit was the smallest whereby we could be reasonably confident that the variations were real. The two-pixel limit, the length of the breathing cycle, and the need to observe both artery and vein simultaneously limited the number of fetal tracings we could analyze to 11. This two-pixel limit was also adopted to assess variations in arterial velocities in the 11 fetuses. We identified critical points, or extrema, in umbilical venous flow velocities, namely relative minima and maxima, and measured all umbilical arterial diastolic and systolic velocities in the breathing cycle being examined. At each critical point we compared the umbilical arterial diastolic and systolic velocities in the prior cardiac cycle with those in the next cycle. We adopted the following criteria to label "next" and "prior" cycles. The "next diastole" was that umbilical arterial velocity that occurred at least one half cardiac cycle after the venous critical point. The "next systole" was that umbilical arterial systolic velocity that immediately followed the next diastole. The prior umbilical arterial diastole and systole occurred in the previous cardiac cycle. An example of the relationships between next diastole, next systole, prior diastole, and prior systole and the venous critical points is displayed in Fig. 1. We noted increases (next diastole greater than prior diastole, next systole greater than prior systole) or decreases (next diastole less than prior diastole, next systole less than prior systole) that were at least two pixels; variations less than two pixels were classified as no change (next diastole equal to prior diastole, next systole equal to prior systole). Pearson's χ^2 test of association (contingency table analysis) was used to determine the significance of the observed correlations between changes in the arterial flow velocities and venous velocity extrema.

Results

Thirty-three fetuses between 25 and 41 weeks' gestation were examined. Coefficients of variation of umbilical arterial systolic and diastolic velocities and heart rate during fetal breathing were obtained in a total of 542 cardiac cycles. No fetus had anomalies, asphyxia, or intrauterine growth retardation at birth (defined as less than 10th percentile body weight by institutional standards). Coefficients of variation measured for each of these three arterial variables are reported in Table I. Umbilical arterial diastolic velocity varied the most and heart rate the least, with diastolic variation about three times that of the heart rate and twice that of the systolic velocity. Coefficients of variation for each variable correlated significantly in individual fetuses (Table I).

Table II summarizes correlations between umbilical arterial and umbilical venous flow in 11 fetuses. Need to image an artery and vein simultaneously for more than one breathing cycle and to have a minimum two-pixel venous velocity variation restricted the number of fetuses we could examine for this correlation to these 11. Simultaneous measurements of next diastole and prior diastole were possible at 48 venous minima and 45 venous maxima, a total of 93 critical points. Umbilical arterial systolic velocities were somewhat more difficult to measure accurately; however, we were able to compare next systole and prior systole at 44 venous minima and 40 maxima, a total of 84 critical points.

Table II reveals a strong correlation between umbilical arterial and umbilical venous flow. After a minimum velocity in the umbilical vein, both diastolic and systolic umbilical arterial velocities tended to increase significantly, and after a maximum velocity in the umbilical vein, both diastolic and systolic umbilical arterial

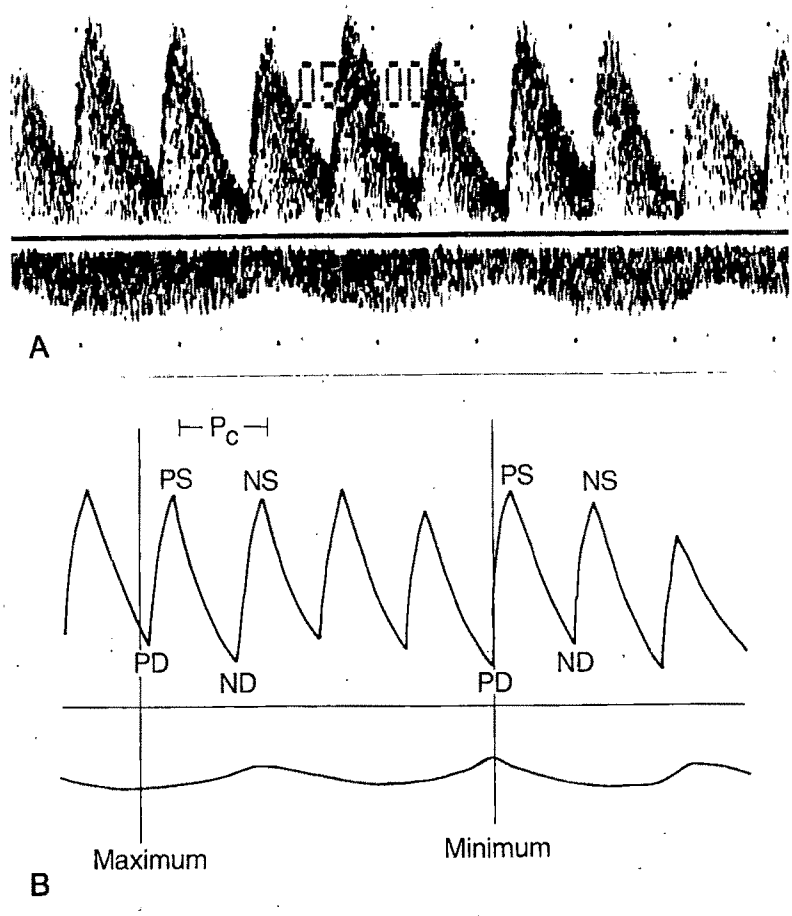


Fig. 1. A, Umbilical arterial and venous Doppler flow velocity waveforms during episode of fetal breathing. Variation in venous velocity indicates fetal respiratory changes. **B,** Diagrammatic representation of Doppler flow velocity waveforms in A, showing relationships between next diastole *(ND)*, prior diastole *(PD)*, next systole *(NS)*, and prior systole *(PS)* around maximum and minimum in venous velocity. Next diastole refers to next diastole that occurs at least one half cardiac cycle *(P_c)* after venous extremum. Next systole is systole that follows next diastole. Prior diastole and prior systole occur one cardiac cycle before next diastole and next systole, respectively. Note that next diastole is less than or equal to prior diastole and next systole is less than or equal to prior systole after venous maximum velocity, and that next diastole is greater than or equal to prior diastole and next systole is greater than or equal to prior systole after venous minimum velocity. *Horizontal dots* are 0.5 seconds apart.

Table I. Coefficients of variation in heart rate and umbilical arterial diastolic and systolic velocities during fetal breathing

	Coefficients of variation (%)
Heart rate	5.0 ± 1.8
Diastolic velocity	16.0 ± 5.0*
Systolic velocity	7.8 ± 2.4*

Heart rate vs diastolic velocity, $r = 0.58$; heart rate vs systolic velocity, $r = 0.58$; diastolic velocity vs systolic velocity, $r = 0.57$; $p < 0.001$ for all r values.

*p Value < 0.001 compared with heart rate.

velocities decreased significantly. The association was especially strong for umbilical arterial diastolic velocity (Fig. 1). There were no instances after an umbilical venous minimum in which umbilical arterial diastole decreased by at least two pixels, and no instances after an umbilical venous maximum in which umbilical arterial diastolic velocity increased by this amount. However, an increase in umbilical arterial velocity after a minimum in venous velocity, and a decrease in umbilical arterial velocity after a maximum in venous velocity were not guaranteed: the umbilical arterial diastolic change was under the two-pixel limit for 32% of the critical points. Likewise, the umbilical arterial systolic change was negligible for 43%. In fact, these instances reflect our findings that the overall variation in the umbilical arterial waveform during fetal breathing was not great, but was larger for diastole than for systole.

We assessed the significance of these correlations with a χ^2 test of association. First we tested the null hypothesis that the outcomes in which next diastole was less than prior diastole, next diastole was greater than prior

Table II. Observed correlation frequencies between umbilical venous velocity maxima and minima and umbilical arterial diastolic and systolic velocities

	Diastole			*Systole*		
	ND > PD	*ND < PD*	*ND = PD*	*NS > PS*	*NS < PS*	*NS = PS*
Minimum	37	0	11	24	5	15
Maximum	0	26	19	4	15	21

Diastole $\chi^2 = 65.0$; $p < 0.001$; systole $\chi^2 = 20.1$; $p < 0.001$.
ND, Next diastole; *PD*, prior diastole; *NS*, next systole; *PS*, prior systole.

diastole, and next diastole was equal to prior diastole were independent of being at an umbilical venous velocity minimum or maximum. We found that for umbilical arterial diastole, $\chi^2 = 65.0$. The probability of finding a χ^2 this large if the null hypothesis is correct was <0.001. The corresponding test of the null hypothesis for umbilical arterial systole gives a $\chi^2 = 20.1$ and a significance also >99.9% ($p < 0.001$).

We concluded that umbilical venous and arterial flow were not independent of each other. The trend was for umbilical arterial diastolic and systolic velocity to increase after a minimum in umbilical venous flow and to decrease after a maximum in umbilical venous flow.

Comment

We showed that heart rate and diastolic and systolic Doppler velocities in the umbilical artery vary during fetal breathing. The largest variation during breathing occurred during diastolic umbilical arterial flow. This finding of larger variations during the diastolic portion of the umbilical artery waveform, during forward flow into the placenta, suggests that compensation for alterations in blood flow related to intravascular pressure changes occurs primarily during diastole.

Furthermore, umbilical artery diastolic and systolic velocity variations correlate with phases of the breathing cycle, as reflected in the variations in umbilical venous blood flow. After a maximum in the umbilical venous velocity, both diastolic and systolic umbilical arterial velocity tended to decrease, whereas after a minimum in the umbilical venous velocity, both umbilical arterial components tended to increase. The significance of these correlations was >99.9% ($p < 0.001$) for both diastole and systole in the umbilical artery.

These results suggest that the circulation between fetus and placenta should be viewed as an interdependent system. Changes in velocity of venous blood flow returning to the fetus affect the arterial flow velocity returning to the placenta and vice versa. During breathing, the fetal intrathoracic pressure varies, and studies in instrumented fetal lambs showed that decreases in intratracheal pressure are associated with increases in blood flow in the superior and inferior vena cava.[8]

Because the velocity of umbilical venous blood flow is determined by the pressure difference between placenta and fetus, a change in intrathoracic pressure will affect umbilical venous flow. We speculate that an alteration in umbilical venous flow affects both ventricular and placental filling. When umbilical venous flow is increasing (after a minimum in umbilical venous velocity), the ventricles fill more, and by the Frank-Starling mechanism, the heart ejects more blood with a resultant increase in umbilical arterial systolic velocity.[7] Furthermore, as more blood empties from the placenta into the fetus (again after a minimum in umbilical venous velocity), the placental blood volume (and presumably pressure) will decrease. Umbilical arterial diastolic flow into the placenta then increases. After a maximum in venous velocity, umbilical venous flow velocity decreases, and umbilical arterial systolic velocity decreases because of decreased ventricular filling. Umbilical arterial diastolic velocity also decreases, probably as a result of the more-filled state of the placenta and its consequent higher pressure.

Secondary effects may serve to moderate the overall variation in umbilical Doppler velocities. For instance, increased umbilical arterial diastolic flow fills the placenta and increases its pressure, which should in turn slow umbilical arterial diastolic flow. In addition, increased umbilical venous flow empties the placenta and decreases its pressure, which in turn slows the umbilical venous flow because flow results from a differential in pressure.

Variations of umbilical arterial systolic and diastolic velocities of 5.4% to 21% (Table I) were apparently well-tolerated, inasmuch as all fetuses were normal at birth. The fact that variations in umbilical arterial blood flow velocities are associated with variations in umbilical venous blood flow velocities suggests that the placenta must be viewed not only as an organ of resistance, but also as an organ through which intrathoracic (and intracardiac) pressure changes may be transmitted.

In summary, human fetal umbilical arterial velocities vary during fetal breathing, with the largest variation during diastole. Furthermore, human fetal umbilical venous and arterial Doppler velocities are interdependent, as shown by changes that occur during presumed variations in intrathoracic pressure with fetal breathing. These changes in velocity are compatible with pressure transmission through the placenta, and, in addition, confirm the existence of the Frank-Starling mechanism in the fetal heart. The interdependency of umbilical

venous and arterial velocities must be considered in the interpretation of the changes in umbilical arterial systolic and diastolic velocities reported in fetuses with growth retardation.

REFERENCES

1. Trudinger BJ, Giles WB, Cook CM. Flow velocity waveforms in the maternal uteroplacental and fetal umbilical placental circulation. AM J OBSTET GYNECOL 1985;152:155-63.
2. Schulman H. The clinical implications of Doppler ultrasound analysis of the uterine and umbilical arteries. AM J OBSTET GYNECOL 1987;156:889-93.
3. Trudinger BJ. The umbilical circulation. Semin Perinatol 1987;11:311-21.
4. Divon MY, Guidetti DA. Doppler ultrasound—a simple technique for identification of respiratory sinus arrhythmia (RSA) in the human fetus. In: Proceedings of the 34th Annual Meeting of the Society for Gynecologic Investigation, March 18-21, 1987. Atlanta, Georgia: Society for Gynecologic Investigation, 1987:215.
5. Hoskins PR, McDicken WN, Johnstone FD, White G, Haddad NG, Chambers SE. Determination of the presence of fetal apnea using umbilical artery and umbilical vein Doppler waveforms. Ultrasound Med Biol 1988;14:589-92.
6. Reed KL, Anderson CF, Shenker L. Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow. AM J OBSTET GYNECOL 1987;157:774-9.
7. Reed KL, Sahn DJ, Marx GR, Anderson CF, Shenker L. Cardiac Doppler flows during fetal arrhythmias: physiologic consequences. Obstet Gynecol 1987;70:1-6.
8. Reuss ML, Rudolph AM, Dae MW. Phasic blood flow patterns in the superior and inferior venae cavae and umbilical vein of fetal sheep. AM J OBSTET GYNECOL 1983;145:70-8.

Potential prenatal predictions of Down syndrome: A statistical analysis

William F. O'Brien, MD, Robert A. Knuppel, MD, MPH, Carol Torres, BS, and Deborah Sternlicht, RN, MS

Tampa, Florida

To determine the feasibility of combining several screening tests for the prenatal detection of Down syndrome, we evaluated the potential relationship among three proposed predictors. We determined the concentration of chorionic gonadotropin in frozen serum samples from women of known maternal age and weight, fetal biparietal diameter, and femur length, and α-fetoprotein concentration. When corrected for gestational age and maternal weight, the potential predictors were independent, except for a slight correlation (r = 0.10) between maternal serum α-fetoprotein and maternal serum human chorionic gonadotropin. Both maternal serum human chorionic gonadotropin and biparietal diameter/femur length demonstrated an approximately log-normal distribution similar to maternal serum α-fetoprotein. Therefore it is scientifically sound to use any or all of these variables in combination for the identification of pregnancies at increased risk for Down syndrome. (AM J OBSTET GYNECOL 1990;163:1796-8.)

Key words: Prenatal diagnosis, Down syndrome, α-fetoprotein

Because recognition of the association of fetal Down syndrome with a low maternal serum α-fetoprotein (AFP), a number of investigators have demonstrated the feasibility of the combined use of maternal age and maternal serum AFP concentration for the selection of women at increased risk for Down syndrome. In addition to maternal serum AFP, several potential markers for Down syndrome have been proposed. Of these, the concentration of human chorionic gonadotropin (hCG) in maternal serum[1] and the fetal biparietal diameter/femur length ratio[2] seem to be the most promising. Because the bayesian analysis of risk requires that assignment of final risk can only be achieved if the indicators of relative risk are independent, we sought to determine whether these proposed markers were independent of each other and of maternal age, and to examine the distribution of values in normal pregnancies.

From the Department of Obstetrics and Gynecology, University of South Florida College of Medicine.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: William F. O'Brien, MD, Department of Obstetrics and Gynecology, University of South Florida, College of Medicine, Suite 500, 4 Columbia Dr., Tampa, FL 33606.
6/6/24703

Material and methods

Specimens were obtained from frozen samples stored at −70° C. These samples had been submitted to the

University of South Florida Regional Screening Program from Jan. 1, 1987, through Dec. 31, 1988. The following criteria were required for choice of samples: ultrasonographic measurement of biparietal diameter and femur length performed on the day of serum sampling, menstrual dating agreeing within 10 days of ultrasonographic dating, and gestational age between 15 and 19 completed weeks. When these criteria were met in more than 200 samples for a given gestational week, every other sample was chosen. Maternal age and ultrasonographic measurements, as well as assurance that the infant was phenotypically normal, were provided by the referring physician. Both maternal serum AFP and hCG concentrations were measured by enzyme-linked immunosorbent assay with commercially available kits (Abbott Laboratories, Abbott Park, Ill.). Determinations were made in duplicate and the coefficient of variation was required to be <10%. For convenience in comparison, results for maternal serum AFP were expressed as international units per ml and maternal serum hCG was expressed as international units per liter.

Samples were grouped according to completed gestational weeks and weekly medians for maternal serum AFP, and maternal serum hCG, and biparietal diameter/femur length ratios were determined. Smoothed medians were then calculated by weighted linear regression. Adjustment of maternal serum AFP and maternal serum hCG for variation in maternal weight was by regression of the nonadjusted multiples of the median with reported maternal weight. All analyses were accomplished with the SAS statistical packages (SAS Institute, Cary, N. C.).

Results

There was no correlation between maternal age and any of the three variables, either as uncorrected values or after transformation. Initial analysis confirmed the expected correlations between gestational age and maternal serum AFP ($r = 0.23$), maternal serum hCG ($r = -0.25$), and biparietal diameter/femur length ratio ($r = -0.33$). Smoothed weekly medians for each variable were generated (Table I) and values were transformed into multiples of the median. This transformation nullified an association with gestational age. However, a significant positive correlation remained between maternal serum hCG and maternal serum AFP ($r = 0.15$). Because maternal serum AFP ($r = -0.18$) and maternal serum hCG ($r = -0.23$) were both varied inversely with maternal weight, both were subsequently expressed as weight-adjusted multiples of the median. After this transformation, there was no longer a correlation with maternal weight, but a small ($r = 0.10$) but statistically significant correlation between maternal serum AFP and maternal serum hCG remained (Table II). Logarithms of maternal serum hCG multiples of the median closely approximated gaussian distribution with a Shapiro-Wilk statistic value of 0.98, whereas maternal serum AFP achieved a value of 0.96 (values may range from 0 to 1.0; 1.0 indicates completely normal distribution). The distribution of biparietal diameter/femur length with a value of 0.88 differed more from a completely normal distribution value of 0.88, but conformed sufficiently to allow clinical usage.

Table I. Smoothed weekly medians for possible screening parameters

Week	*n*	*Maternal serum AFP*	*Maternal serum hCG*	*BPD/FL*
15	76	24.1	25.0	1.73
16	146	29.4	21.0	1.66
17	130	34.7	17.0	1.60
18	120	40.4	12.7	1.53

BPD/FL, Fetal biparietal diameter/femur length ratio.

Table II. Relationship between predictors of Down syndrome

Variable 1	*Variable 2*	*r*	*p*
Maternal serum hCG	BPD/FL	−0.04	0.37
Maternal serum AFP	BPD/FL	0.06	0.16
Maternal serum hCG	Age	0.05	0.22
Maternal serum AFP	Age	0.03	0.45
Maternal serum hCG	Maternal serum AFP	0.10	0.02

BPD/FL, Fetal biparietal diameter/femur length ratio; *Age,* maternal age; *r,* Spearman Rank correlation coefficient; *p,* probability.

Comment

The traditional method of patient selection for Down syndrome testing by amniocentesis has been maternal age. Unfortunately, with use of this criterion alone only 20% to 30% of women with a fetus with Down syndrome are offered amniocentesis.[5] After the initial report that women carrying fetuses with Down syndrome had lower than average maternal serum AFP values,[1] this observation was confirmed and tables allowing the use of combined maternal age and maternal serum AFP concentration to estimate the risk of Down syndrome were published.[5] Several recent reports[6, 7] have confirmed the value of combining maternal age with maternal serum AFP values to select a population of women younger than the traditional cutoff age with a risk similar to that of older women. Unfortunately, even with the adjunctive, use of maternal serum AFP detection of a significantly increased proportion of fetuses with Down syndrome requires a high false-positive rate.[8]

The potential of using a combination of noninvasive measures to increase the sensitivity or decrease the false positivity of Down syndrome screening is of great cur-

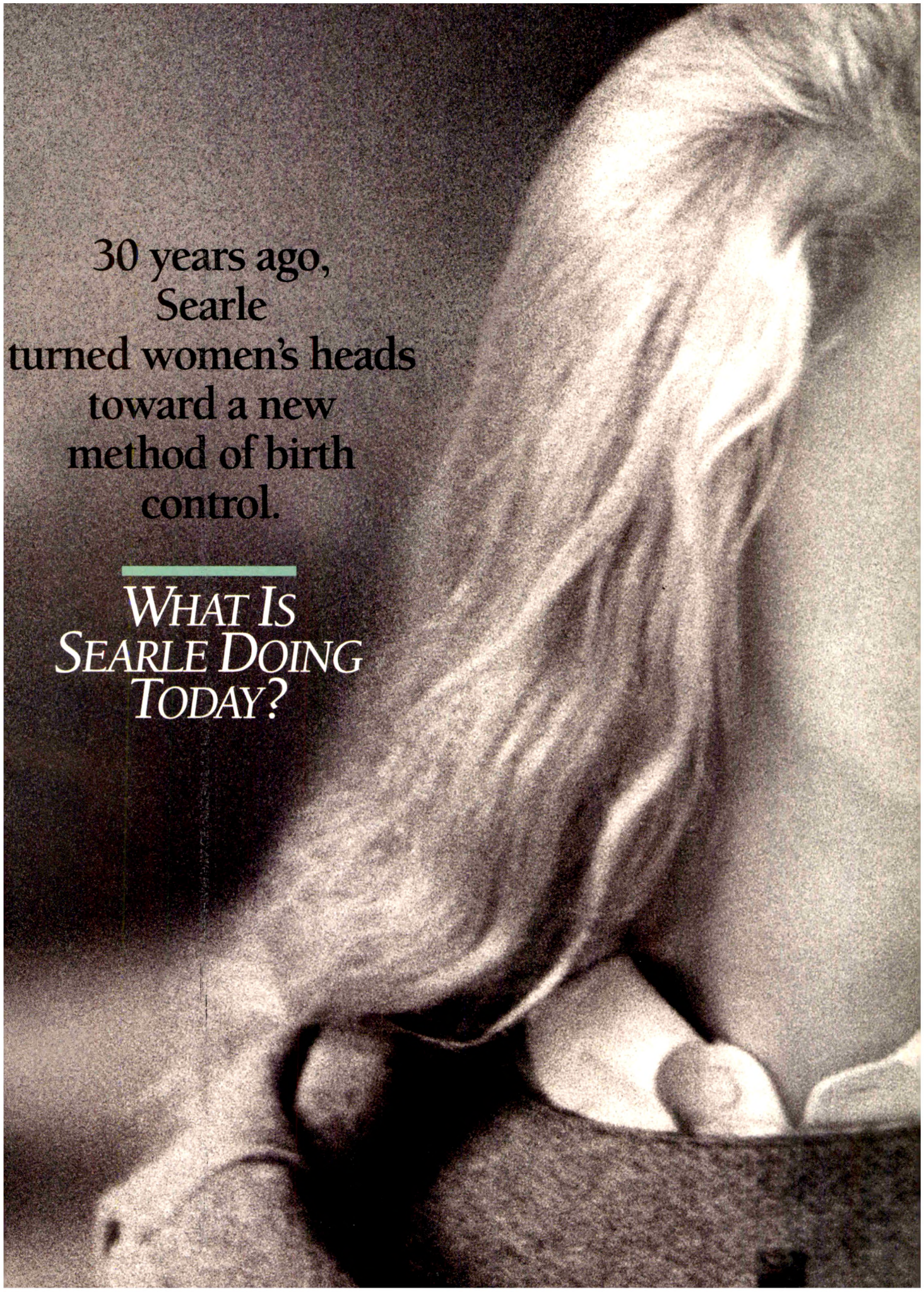
rent interest. Of the possible candidates for such a role, the biparietal diameter/femur length ratio and maternal serum hCG appear to be the most promising. Inclusion of the biparietal diameter/femur length ratio is unlikely to result in a significant increase in cost, because most programs require ultrasonographic confirmation of dating before final recommendation for amniocentesis. Unlike maternal serum AFP, which is measured primarily to screen for open neural tube defects, determination of maternal serum hGC would increase screening costs. Currently, however, it appears to be the most likely candidate as a second screen since it performs more efficiently than measurement of unconjugated estriol at similar cost.

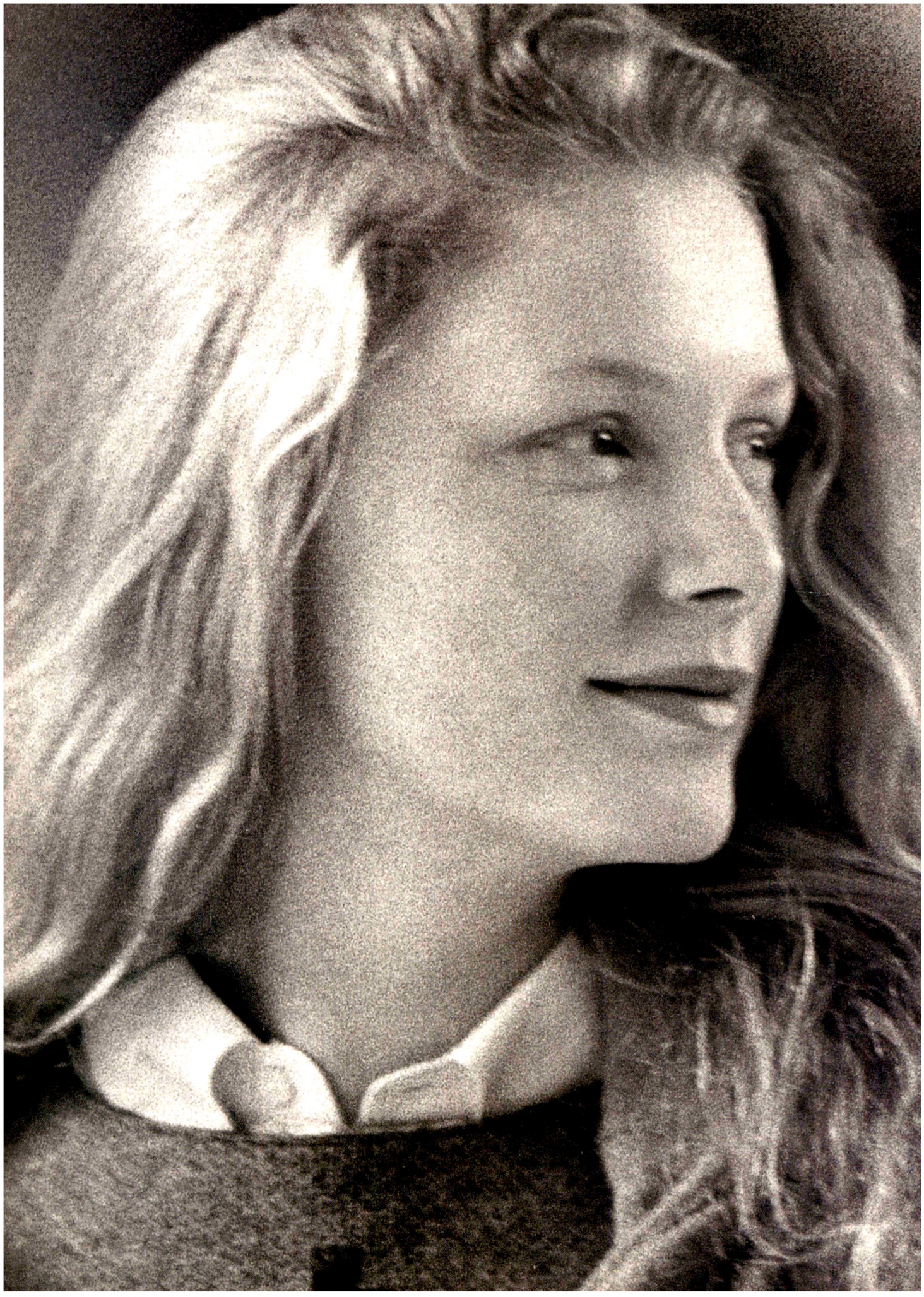
In this study we demonstrated that, after adjustment for gestational age and maternal weight, maternal serum AFP, maternal serum hCG, and biparietal diameter/femur length ratio are sufficiently independent from each other and from maternal age that they may be combined in a bayesian estimate of relative and final risk. The correlation between maternal serum AFP and maternal serum hCG found in our sample before adjustment for maternal weight (0.15) is similar to the value (0.14) reported by Wald et al.[9] in samples for which maternal weight was not available. However, maternal weight was only partially responsible for the correlation and it is probable that there is an underlying biologic association. In contrast, the lack of association between biparietal diameter/femur length ratio and either of the biochemical measures assures that the combination of retarded femur growth, increased production of hCG, and deficient production of AFP seen in Down syndrome does not occur in chromosomally normal fetuses.

Although these results are encouraging, care is required before the widespread introduction of maternal serum hCG and biparietal diameter/femur length ratio for Down syndrome screening. First, estimation of relative risk on the basis of the distributions of normal and Down syndrome pregnancies is relatively straightforward, but there is some concern that the distribution of maternal serum AFP values in pregnancies associated with Down syndrome may be dependent on maternal age or on geographic site.[10] The distribution of maternal serum hCG in pregnancies associated with Down syndrome has only been determined in a small number of cases, and similar concerns must be considered. Second, the values for the biparietal diameter/femur length ratios noted in this study are similar to those reported from Boston, but they differ from those generated in New Haven[11] with intermediate results reported from Pittsburgh.[12] Although it is possible that these differences are based on population, it is more likely that they are related to technique.[2] Unlike maternal serum AFP or maternal serum hCG determinations, over which laboratories can exert quality control, most centers rely on ultrasonographic values generated by the referring physician. Significant variations in ultrasonographic technique among participating physicians may obscure the value of biparietal diameter/femur length ratio on screening. Finally, the cost effectiveness and goals of the screening program must be evaluated. Should maternal serum hCG be determined for all subjects or only those found at increased risk by evaluation of maternal age and maternal serum AFP? We have shown that the combination of maternal age, maternal serum AFP, maternal serum hCG, and biparietal diameter/femur length ratio in the prediction of Down syndrome risk may be feasible. The proper implementation of such a program requires further study.

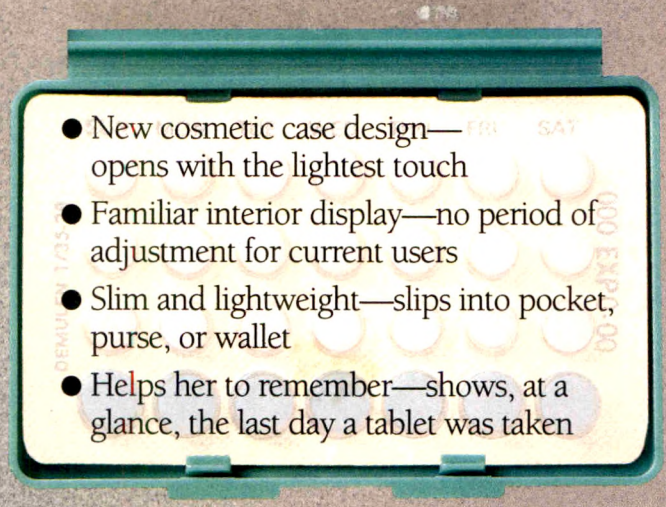
REFERENCES

1. Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn 1987;7:623-30.
2. Lockwood C, Benacerraf B, Krinsky A, et al. A sonographic screening method for Down syndrome. AM J OBSTET GYNECOL 1987;157:803-8.
3. Adams M, Erikson J, Layder P, Oakley GP. Down's syndrome: recent trend in the United States. JAMA 1981;246:758-60.
4. Merkatz IR, Nitowski HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. AM J OBSTET GYNECOL 1984;148:886-91.
5. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet 1984;1:926-9.
6. DiMaio MS, Baumgarten A, Greenstein RM, Saal HM, Mahoney MJ. Screening for fetal Down's syndrome in pregnancy by measuring maternal serum alpha-fetoprotein levels. N Engl J Med 1987;317:342-6.
7. Palomaki GE, William J, Haddow JE. Combining maternal serum alpha-fetoprotein measurements and age to screen for fetal Down syndrome in pregnant women. AM J OBSTET GYNECOL 1989;160:575-81.
8. Spencer K, Carpenter P. Screening for Down's syndrome using alpha-fetoprotein: a retrospective study indicating caution. Br Med J 1985;290:1940-3.
9. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. Br Med J 1988;297:883-7.
10. Hook EB. Variability in predicted rates of Down syndrome associated with elevated maternal serum alpha-fetoprotein levels in older women. Am J Hum Genet 1988;43:160-4.
11. Lockwood C, Benacerraf B, Krinsky A, et al. A sonographic screening method for Down syndrome. AM J OBSTET GYNECOL 1987;157:803-8.
12. Hill LM, Guzick D, Belfar HL, Hixson J, Rivello D, Rusnak J. The current role of sonography in the detection of Down syndrome. Obstet Gynecol 1989;74:620-3.


30 years ago,
Searle
turned women's heads
toward a new
method of birth
control.
WHAT IS
SEARLE DOING
TODAY?



MAKING A GREAT CASE FOR DEMULEN® 1/35



- New cosmetic case design—opens with the lightest touch
- Familiar interior display—no period of adjustment for current users
- Slim and lightweight—slips into pocket, purse, or wallet
- Helps her to remember—shows, at a glance, the last day a tablet was taken

THE CASE FOR PRESCRIBING DEMULEN 1/35

- Low-dose formulation
- Minimal lipid impact
- Low incidence of some common androgenic side effects, such as acne, oily skin, and hirsutism
- Unique progestogen: ethynodiol diacetate
- No generic substitute available
- Covered by the Patient Promise® program

  If you prescribe Demulen 1/35, or any other Searle product, and do not achieve the desired therapeutic benefits, at any time, Searle will refund 100% of your patient's out-of-pocket cost for her most recent prescription for that product, *a full refund directly to your patient.*

DEMULEN® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Also available in the new Compack®
DEMULEN® 1/50 (ethynodiol diacetate 1 mg, ethinyl estradiol 50 mcg)

Please see adjoining page for a brief summary of prescribing information.

The physician should become familiar with the prescribing information (labeling) for this product, which discusses reported risks, such as thromboembolic disorders (including strokes and heart attacks), risk of estrogen dose, and hepatic lesions, as well as the need to monitor patients for early symptoms of any diseases or medical conditions, so use can be discontinued when appropriate. A brief summary of the prescribing information accompanies this advertisement.

SEARLE 30 YEARS EXPERIENCE IN ORAL CONTRACEPTION

© 1990, G.D. Searle & Co.

A90DE3808T

DEMULEN® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

BRIEF SUMMARY

Demulen® 1/35: Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients. **Demulen® 1/50:** Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients.

Indication and usage: For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk:* Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen.

Contraindications: Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products.

Warnings:

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking is a major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. Latter group also had more venous TE. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. Decreased HDL, which relates to increased myocardial ischemia, occurs with high progestational activity. Consider amount of both steroids in choosing OC. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

Be alert to earliest manifestations of TE disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. RCGP reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. Varicose veins have little effect on development of deep vein thrombosis. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. Estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased enc cancer with combination or progestogen-only OCs. Studies show no increased breast cancer with OCs or estrogens. I greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greate breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrog and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with c factors and in various subgroups. Decrease in benign breast tumors in OC users is well known. Some studies si increased risk of cervical dysplasia, CIN, erosion, and carcinoma in long-term users. Cervical microglandular hyperp been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OC surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonf causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history c cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical d **4.** *Hepatic adenomas, etc,* have been associated with OCs. In 1 study OC formulations with high "hormonal potenc associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemope in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long- users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdomi tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. R and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may b Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur with **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring:* Estrogens or progestogens dur pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens m increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. F 90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES- daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defec estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VAC vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by male was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal wi pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardic defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,00 compared to 7.8 for those unexposed (P<.05). Female hormone exposure in utero was associated with testicular anc cancer. More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence prog are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregn post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstra out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods ar rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and OCs. If pregnancy is confirmed, stop OC and apprise patient of potential fetal risks and discuss continuation of pr Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnan **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 appeared in 6-12 months' use in another. Bile duct cancer has been reported. **7.** *Carbohydrate and lipid metabolism:* D glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Trigl total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but i with longer exposure; in the 5th year it is 2½–3 times the first year. Age is strongly correlated with hypertension Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pr familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during m cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardic disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of mig development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluatin **10.** *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for disconti BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleec out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to i estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenc secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuin with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolong occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been as with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio o to intrauterine pregnancies is no higher than in nonusers. **12.** *Breast feeding:* OCs may decrease quantity and quality A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable d in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Prec** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pel BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy i risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dy and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; mega anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pa of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; i prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; i thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, unalte T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, trig phospholipid; reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonge on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask climacteric. Cervical chlamydial and gonorrheal prevalence is increased; do not assume OC protects against chlamy HIV seropositivity was associated with OC use. *Information for patients.* See patient labeling. *Drug interactions.* OC effec may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloram sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, an tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetam **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombo and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. evidence of association between the following and OCs, though confirmatory studies have not been done: m thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during a use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enla secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, ch jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or ref premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, par nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multi nodosum, hemorrhagic eruption, herpes gestationis, hidradenitis suppurativa, hemolytic uremia, malignant hype impaired renal function, acute renal failure, sometimes irreversible, itching, vaginitis, porphyria, PVCs, abnorm pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythe rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant m endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been repor large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: C prescribing information should be consulted prior to use and is available on request.**

5/14/90 • P90

SEARLE

G.D. Searle & Co.
Box 5110, Chicago, IL 60680

Address medical inquiries to:
G.D. Searle & Co.
Medical & Scientific Information Departm
4901 Searle Parkway
Skokie, IL 60077

The course of labor after endurance exercise during pregnancy

James F. Clapp III, MD
Burlington, Vermont

This study was designed to test the hypothesis that continuation of a regular running or aerobics program, or both, during the latter half of pregnancy would have a negative effect on the course and outcome of labor. The onset, course, and outcome of labor were independently monitored in 131 well-conditioned recreational athletes who had an uneventful first half of pregnancy. Daily exercise performance was quantitated before conception and throughout pregnancy. Comparisons were made between the 87 women who continued to exercise regularly at or above 50% of their preconceptional level throughout pregnancy and the 44 who discontinued their regular exercise regimen before the end of the first trimester. The incidence of preterm labor was similar in the two groups (9%). Labor began significantly earlier in the exercise group (277 ± 6 vs 282 ± 6 days). The women who continued to exercise had a lower incidence of abdominal (6% vs 30%) and vaginal (6% vs 20%) operative delivery, and active labor was shorter (264 ± 149 vs 382 ± 275 min) in those who were delivered vaginally. Finally, clinical evidence of acute fetal stress (meconium, fetal heart pattern, and Apgar score) was less frequent in the exercise group (50% vs 26%), although birth weight was reduced (3369 ± 318 vs 3776 ± 401 gm). These data negate the initial hypothesis and indicate that, in well-conditioned women who regularly perform aerobics or run, continuation of these exercise regimens has a beneficial effect on the course and outcome of labor. (AM J OBSTET GYNECOL 1990;163:1799-805.)

Key words: Exercise, pregnancy, labor

It is likely that regular physical activity, as well as the improved physical fitness that accompanies it, has a beneficial effect on the course and outcome of labor. However, a direct assessment of this relationship is difficult because of the confounding effects of the multiple exercise variables involved and the other maternal, uterine, fetal, and obstetric factors that are known to influence labor. Thus it is not surprising that the reports dealing with the impact of exercise during pregnancy on labor have been divergent with some reporting benefit[1,2] and others either no effect[3-6] or a detrimental one.[7,8]

The purpose of this study was to provide a definitive answer to the question, does continuation of a regular exercise regimen throughout pregnancy influence the course and outcome of labor? In an attempt to control the multiple variables involved, the current study used a prospective design and was limited to well-conditioned recreational athletes who performed one of two forms of high-intensity exercise (running or aerobics) in which a large fraction of the body's muscle mass is used against gravity for relatively prolonged periods of time. The experimental design also included a relatively large sample size, a matched populace of recreational athletes who discontinued exercise, daily monitoring of exercise performance before and during pregnancy, and on-site, independent monitoring of the course and outcome of labor. The hypothesis tested was that when a regular running or aerobics regimen is continued at or above 50% of preconceptional levels throughout pregnancy it has a negative impact on the course and outcome of labor. The objective outcome parameters included indices of labor onset, management, course, complications, outcome, and fetal stress.

From the Department of Obstetrics and Gynecology, The University of Vermont College of Medicine.
Supported in part by National Institutes of Health grant HD21268 and grant 6-464 from the March of Dimes Birth Defects Foundation.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: James F. Clapp III, MD, Physiology Laboratory, Department of Obstetrics and Gynecology MetroHealth Medical Center, 3395 Scranton Road, Cleveland, OH 44109.
6/6/24724

Material and methods

Between July 1983 and February 1990, we prospectively monitored the spontaneous exercise performance of 67 recreational runners and 64 aerobic dancers who, with the exception of preterm labor, had clinically normal pregnancies, with labor and delivery under surveillance by study personnel. All subjects had been exercising regularly for at least 6 months before conception, three or more times each week for at least 30 minutes each session at an intensity >50% of their individual maximum capacity. Written informed consent was obtained from each subject at the time of enrollment. Because of very real human subject concerns and an unwillingness on the part of many subjects to change their specific exercise regimen, the subjects were not randomized prospectively to different regimens. Rather, they were divided into two groups on the basis of their spontaneous level of exercise perfor-

mance during pregnancy. The 46 runners and 41 aerobic dancers who continued to exercise at or above 50% of their preconceptional performance level throughout the 10 lunar months of pregnancy formed the continued exercise group. The 21 runners and 23 aerobic dancers who spontaneously stopped their regular exercise regimen by the end of the first trimester formed the discontinued exercise or reference group.

To quantitate each individual's exercise, we measured maximum oxygen consumption before conception and the relationship between heart rate and oxygen consumption during graded exercise in the laboratory before conception and every 6 to 8 weeks during pregnancy. Concurrent field heart rate data and the time spent in exercise were gathered with a portable telemetry system, and the data were used to calculate overall weekly exercise performance. This was quantitated by means of the training effect method and was expressed as a duration-intensity index (product of weekly duration and average weekly intensity).[9]

Pregnancy was confirmed within a few days of missed menses (β-human chorionic gonadotropin level) and dates were confirmed by early ultrasonography 53 to 60 days after the last menstrual period. With the exception of preterm labor, the pregnancies were all clinically normal and the course and outcome of each labor were monitored by an independent observer who was present but was not involved in the subject's clinical care.

The onset of labor was defined as the onset of regular uterine contractions followed by progressive cervical change. Preterm labor was defined as the onset of labor before 263 days' gestation (<37½ weeks of gestation completed), and premature rupture of the membranes was defined as the onset of persistent vaginal fluid leakage before the onset of subjective uterine activity that was confirmed on examination by an alkaline pH and ferning of the fluid. The start of the active phase of labor was set at 4 cm of cervical dilation with regular uterine contractions, and women who were further advanced in labor at the time of initial evaluation were not included in the study. An abnormal fetal heart pattern was defined as one that initiated medical intervention and the Apgar score was assigned by the delivery room nurse or pediatrician in attendance.

Significant between-group differences for normally distributed parameters were detected with the unpaired Student t test. Differences in exercise performance were assessed with the Wilcoxon–Mann-Whitney test. Between- and within-group differences in incidence were detected by means of the χ^2 statistic with the Yates correction for small groups. Regression analysis was used to detect any within-group effect of weight gain, exercise performance, preconceptional weight, and parity on numeric parameters. Significance was set at the 0.05 level.

Results

Maternal characteristics. The subjects were all white, were middle or upper socioeconomic class, and worked part-time or full-time. None smoked, alcohol intake was limited (maximum recorded was four glasses of beer per week), and all subjects ate an adequate caloric, well-balanced diet that was low in simple sugar and high in complex carbohydrate. They were all in excellent health and averaged 31 years of age (range 24 to 38). Preconceptional weight and height ranged between 42.3 and 78.4 kg and between 155 and 185 cm, respectively. Most (128/131) had a normal menstrual pattern; 52% of the populace were nulliparous and 47% were primiparous. Two subjects were secundiparous. The only obstetric risk factors included one subject with a history of diethylstilbestrol exposure, two with a history of bulimia in college, and one with a prior preterm delivery. Data on family obstetric history were not obtained, but there were no between-group differences in the distribution of birth orders, birth weights, or route of delivery of the subjects themselves.

All subjects were fit as assessed by preconceptional morning pulse rate (range 48 to 62 beats/min), maximal oxygen consumption (range 43 to 68 ml/kg/min), weight for height (range of ponderal index 1.06 to 1.38), and percent body fat (range 10% to 24%). When referenced to current normative standards,[10] all subjects had maximum oxygen consumptions in the upper 15% of the overall populace with an average of 54.3 ml/kg/min.

There were no significance between-group differences in any of these demographic, morphometric, lifestyle, and obstetric variables or in the four objective indices of fitness. In fact, the only two discernible between-group differences were that the women who discontinued their regular exercise regimen in early pregnancy had an overall level of exercise performance before conception that averaged 25% less than that of the women who continued their regular regimen throughout pregnancy (Table I) and their average pregnancy weight gain (17.2 kg) was 3.6 kg greater than that of those who continued exercise (13.6 kg).

Maternal exercise performance. Before conception running performance ranged between 14 and 68 km each week at a pace between 3.9 and 6.1 min/km and an intensity ranging between 51% and 83% of maximum. Participation in aerobics ranged between 3 and 11 sessions each week at an intensity between 54% and 90% of maximum during the 25- to 30-minute high-intensity phase of the workout. The mean group pulse rate was 166 beats/min during both aerobics and running, but there was a great deal of individual variation with individual average exercise pulse rates ranging between 122 and 190 beats/min. The relationship between pulse rate and exercise intensity was extremely variable such that a pulse rate of 140 beats/min for

Table I. Maternal exercise performance

	Control (N = 44)	*Exercise (N = 87)*	*Significance*
Duration-intensity index before pregnancy	9840 ± 303	13,070 ± 332	0.01
During pregnancy* (%)	14 ± 17	68 ± 16	0.01
Last half of pregnancy* (%)	5 ± 10	65 ± 17	0.01
9-10 mo of pregnancy* (%)	2 ± 3	60 ± 18	0.01

Values are mean ± SD.
*Percent of prepregnancy value.

different individuals represented an oxygen consumption that ranged between 42% and 73% of measured maximum capacity.

During the first half of pregnancy the intensity of exercise was maintained at or near preconceptional levels (51% to 90% of maximum) in the continued exercise group. However there was an average decrease in running distance and number of aerobics classes to 29 km/wk and 4.8 classes per week, resulting in a 23% average reduction in the time spent in exercise during the first half of pregnancy. The relationship between exercise intensity and pulse rate became more variable. At 8 and 16 weeks a pulse rate of 140 beats/min for different individuals represented an oxygen consumption that ranged between 31% and 76% of preconceptional maximum capacity. During the latter half of pregnancy the range of intensity decreased to 50% to 76% of maximum capacity, but the range of the time spent in exercise increased (40% to 160% of preconceptional duration). As a result the average running distance and overall aerobics effort remained stable. Again there was wide individual variation in both pulse rate and its relationship to exercise intensity. Individual average pulse rates during exercise ranged between 110 and 187 beats/min, and a rate of 140 beats/min for different individuals represented an oxygen consumption that ranged between 35% and 68% of preconceptional measured maximum capacity. Thus during pregnancy the slope of the relationship between individual pulse rate and exercise intensity, measured as a percentage of preconceptional maximum absolute oxygen consumption, changed in different directions at different time points in pregnancy, and individual average pulse rates, which ranged between 110 and 195 during exercise in pregnancy, were not by themselves an accurate index of individual exercise intensity. The individual range of overall exercise performance for the entire pregnancy ranged between 50% and 109% of preconceptional levels. For the latter half of pregnancy the range in individual overall performance was 50% to 122% of preconceptional levels. Mean values for the entire group are listed in Table I for the entire pregnancy, the last 5 months, and last 2 months.

Although the women who discontinued regular exercise had an overall preconceptional performance level that averaged 25% less than that of those who continued, their overall level of performance still represented an average of 26 km/week for the runners and an average of four or five classes per week for those who performed aerobics at similar intensities. The majority of these women (n = 37) spontaneously discontinued exercise in early pregnancy for one of two reasons. First, dealing with the extreme fatigue and nausea of early pregnancy completely abolished their desire to exercise early on, and they simply never prioritized the time to begin their regular exercise regimen again during pregnancy. Second, they could not cope emotionally with the reduction in performance level suggested by their physicians or their own uncertainties concerning the potential impact of the exercise on the pregnancy, which were reinforced at the initial prenatal visit. As a result they simply stopped until after delivery. In the remainder minor injury, job pressures, or paternal concerns played a major role. As a group (Table I) their average level of overall performance fell to 2% to 5% of preconceptional levels by the end of the fifth lunar month.

Onset of labor. As detailed in Table II, continuing the ballistic motion associated with running and aerobics throughout pregnancy did not increase the incidence of either the onset of labor before 37½ weeks of gestation were completed or membrane rupture before the onset of perceived labor. The incidence of spontaneous membrane rupture that eventually necessitated induction of labor was also similar in the two groups. However, the onset of labor at or near term occurred 5 days earlier on average in the women who continued exercise. When examined individually within groups, prepregnancy weight, pregnancy weight gain, parity, preconceptional or pregnant level of exercise performance, and exercise type (aerobics or running) did not have a significant effect on gestational length (all individual R^2 values <0.02).

Obstetric management. As a group the women who continued their regular exercise regimens throughout pregnancy experienced significantly less obstetric intervention (Tables III and IV). This included highly significant reductions in both elective and indicated labor stimulation, episiotomy, and use of epidural anesthetic, as well as both vaginal and abdominal opera-

Table II. Onset of labor

	Control (N = 44)	*Exercise (N = 87)*	*Significance*
Preterm (<day 263)	9% (*n* = 4)	8% (*n* = 7)	NS
Term (mean ± SD)	282 ± 6	277 ± 6	0.01
PROM at term	29% (*n* = 13)	30% (*n* = 26)	NS
Induction of labor	13% (*n* = 6)	13% (*n* = 11)	NS

Actual number of cases is shown in parentheses. *PROM*, Premature rupture of membranes.

Table III. Labor management

Parameter	*Control (N = 44)*	*Exercise (N = 87)*	*Significance*
Artificial rupture of membranes	50% (*n* = 22)	23% (*n* = 20)	0.01
Stimulation for abnormal labor pattern	20% (*n* = 9)	13% (*n* = 11)	0.01
Second stage arrest	14% (*n* = 6)	2% (*n* = 2)	0.01
Epidural anesthetic used	50% (*n* = 22)	28% (*n* = 24)	0.01
Method of delivery			
Spontaneous	50% (*n* = 22)	88% (*n* = 77)	0.01
Forceps	20% (*n* = 9)	6% (*n* = 5)	0.01
Cesarean section	30% (*n* = 13)	6% (*n* = 5)	0.01

Actual number of cases is shown in parentheses.

tive delivery. This was not due to a between-group difference in the number of parous subjects. Rather, it was directly related to a significant increase in the incidence of both protraction disorders and second-stage arrest in the discontinued exercise group, which created a greater need for stimulation and operative intervention. The latter also had a causal role in the increased duration of active labor in the control group. However, when the cases in which cesarean section was the method of delivery were excluded, active labor was still 30% shorter on average in the exercise group, and within both groups parous subjects had significantly shorter labors than nulliparous women. Within the discontinued exercise group there was a significant direct relationship ($R^2 = 0.1103$) between maternal weight gain and the length of labor, and χ^2 analysis indicated that a weight gain in excess of 18 kg was associated with a significant increase in the incidence of both protraction disorders and operative delivery. These relationships were not present in the continued exercise group. Similarly, exercise performance before or during pregnancy and exercise type did not have any additional effect in the exercise-continued group.

Fetal condition and outcome. The parameters evaluated for assessing the fetal condition and outcome included meconium-stained fluid, fetal heart patterns, visual evidence of a nuchal cord at delivery, and Apgar score. Scalp pH was determined infrequently and was always in the normal range. A fetal heart rate pattern was thought to be abnormal only if it initiated a clinical response on the part of the midwife or physician (oxygen, maneuvers to shorten the second stage, abdominal delivery). As can be seen in Table V, each of these four indices of fetal stress occurred more frequently in the discontinued exercise group. Undoubtedly, these occurrences were also a factor in the higher rate of obstetric intervention in the discontinued exercise group. Within-group interactive effects on the four parameters used as evidence of fetal stress were not observed. Finally, birth weight was reduced by approximately 400 gm in the exercise group. This was not related to a between-group difference in the sex of the offspring (exercise continued, 52% male; exercise discontinued, 48% male). Within the discontinued exercise group a birth weight >4000 gm was associated with an increased incidence of protraction disorders and operative delivery. Within the exercise-continued group there was no detectable relationship between birth weight and obstetric intervention with birthweight cutoffs of 3500 and 3750 gm (only one infant weighed >4000 gm). Within the overall populace there was a significant relationship ($r^2 = 0.2768$) between birth weight and pregnancy weight gain. As discussed elsewhere,[11] this correlation is due to the independent effects of exercise on both parameters.

Comment

These data negate the initial hypothesis and clearly indicate that, in physically fit women who performed aerobics or ran on a regular basis before pregnancy, continuing a regular running or aerobics exercise program above a maintenance level throughout pregnancy had a beneficial effect on multiple aspects of the course and outcome of labor. Overall, continued exercise was

Table IV. Management—Vaginal deliveries only

Parameter	*Control (N = 31)*	*Exercise (N = 82)*	*Significance*
Active labor (min) (mean ± SD)	382 ± 275	264 ± 149	0.01
Active first stage (min) (mean ± SD)	302 ± 191	223 ± 134	0.01
Parous	52% (*n* = 16)	53% (*n* = 43)	NS
Forceps use	29% (*n* = 9)	6% (*n* = 5)	0.01
Episiotomy done	81% (*n* = 25)	46% (*n* = 38)	0.01
Epidural anesthetic use	41% (*n* = 13)	29% (*n* = 24)	0.01
Abnormal labor pattern	35% (*n* = 11)	20% (*n* = 16)	0.01

Actual number of cases is shown in parentheses. *Active labor,* 4 cm dilatation to delivery.
Active first stage, 4 to 10 cm dilatation.

Table V. Fetal condition and outcome

Parameter	*Control (N = 44)*	*Exercise (N = 87)*	*Significance*
Meconium in fluid	25% (*n* = 11)	14% (*n* = 12)	0.01
Abnormal heart rate*	25% (*n* = 11)	14% (*n* = 12)	0.01
Nuchal cord	55% (*n* = 24)	26% (*n* = 23)	0.01
1 min Apgar score <7	25% (*n* = 11)	14% (*n* = 12)	0.01
Birth weight (gm) (mean ± SD)	3776 ± 401	3369 ± 318	0.01

Actual number of cases is shown in parentheses.
*Fetal heart rate pattern resulting in physician action.

associated with a shorter labor, less need for obstetric intervention, and fewer signs of fetal stress. Additional correlations suggest that the effect of continued exercise on the management and duration of labor is partially due to its effect on birth weight.[11]

Despite the fact that the 87 women who continued to exercise throughout pregnancy did so at intensities and for time periods well above currently recommended levels, there was no evidence, other than a reduction in birth weight, that the theoretical concerns about these types of exercise in late pregnancy were valid. There was no evidence to support the concern that exercise triggers preterm labor or increases the incidence of membrane rupture before the onset of labor. No cases of abruptio placentae were noted, the incidence of nonvertex presentations was not increased, and clinical evidence of fetal compromise at the onset or during labor was not found. An additional benefit of continuing a regular exercise regimen was the limitation in maternal pregnancy weight gain. However, the fact that this was largely confined to the women runners[10] indicates that it was not a significant determinant of the course and outcome of labor, which did not vary with exercise type.

Although not specifically examined, the rate of progress in and the duration of labor, coupled with the decreased incidence of protraction disorders in the exercise group, suggested that exercise at these levels in late pregnancy may have had a positive effect on cervical ripening and uterine coordinate activity without initiating preterm labor. In fact, three subjects initially qualifying for the exercise group were excluded from analysis because labor was so short that they entered the labor suite at or near full dilatation and were delivered before adequate assessment could be accomplished. These observations, coupled with the low incidence of episiotomy and operative vaginal delivery and an average 40-minute second stage, clearly do not support the concern raised in several earlier publications that these types of exercise may increase the resistance of the perineal tissues.[7,8]

As discussed elsewhere,[11] the difference in birth weight was primarily due to a reduction in fetal fat mass in the offspring of the women who continued a regular exercise regimen throughout late pregnancy. This did not create clinical difficulty in the neonatal period, and if the leanness persists, it may actually turn out to be an advantage in terms of cardiovascular risk in later life. It is conceivable that this difference in birth weight could place the preterm infant at greater risk of morbidity and mortality. However, as the birth weight difference is primarily a difference in fat and the majority of in utero fat deposition occurs after the thirty-fourth week, the difference in birth weight should be minimal in infants born to exercising women before that point in gestation.

Several shortcomings of the current study should be emphasized. First, because of human subject concerns and individual commitment to a chosen exercise regimen, exercise performance during pregnancy was sim-

ply monitored, not randomly assigned. As such, group assignment was essentially self-selected and could potentially introduce bias. However, as detailed above, the two groups were well matched for most demographic, obstetric, morphometric, and life-style factors that are known to affect birthweight and the course and outcome of labor. While this match does not control for some variables such as potential individual differences in hormonal milieu, the discontinued exercise group did provide a valid reference for most of the clinically recognized dependent variables that influence labor. In addition, although the women in the discontinued exercise group performed significantly less exercise each week before conception than those who continued to exercise throughout pregnancy, objectively they were equally fit before pregnancy and, if anything, their level of fitness decreased during pregnancy, which should have had a reverse bias effect on the course and outcome of labor.[2]

While it is possible that the lower level of overall preconceptional exercise performance in the discontinued exercise subgroup could have reflected additional factors that influenced the course and outcome of labor, we could not identify any. Although the performance of this group was relatively lower before conception, it was still significant (27 km/wk or four or five aerobic sessions per week), and they still were extremely fit (average maximum oxygen consumption of 54.2 ml/kg/min) and averaged 150 minutes of high-intensity endurance exercise each week. On the basis of these data we doubt that the difference reflected unrecognized factors that biased labor outcome and speculate that the between-group difference in preconceptional performance simply indicates that women who consistently exercise at a higher overall level are more likely to continue their exercise regimen during pregnancy than women who work at a slightly lower level. Finally, there was no consistent relationship between a subject's intended level of exercise performance during pregnancy and her actual level of performance; i.e., many of the women who planned to continue a vigorous exercise regimen stopped and many women who were hesitant about their ability to maintain an exercise regimen did so. Thus, although the design was not ideal, the reference or discontinued exercise group did not have an easily detectable selection bias of major proportion and the outcomes observed provide the data needed to ethically permit a randomized trial of exercise prescription during pregnancy.

Second, to isolate the effects of regular exercise on the course and outcome of labor, these data were obtained in a select group of well-conditioned recreational athletes who had been regularly performing one of two specific types of exercise for several years. As such, extrapolation of these findings to other women or other exercise regimens would be inappropriate and the results clearly are not applicable to the overall obstetric populace. This restriction is important and is supported by the findings of several well-controlled studies that used less demanding exercise regimens in other groups of women and noted either no or minimal impact on the course and outcome of labor.[4-8] This restriction probably is particularly relevant to the group of women who wish to begin a training regimen for the first time during either the periconceptional period or early pregnancy. Nonetheless, these findings are in accord with the general consensus in the literature that exercise does not adversely affect the course and outcome of labor,[1-6] and the selective nature of the populace studied, along with the monitoring of both the actual level of exercise performance and the course of labor, undoubtedly enhanced our ability to detect the noted differences.

Finally, it is important to emphasize that the 87 women who continued to run and perform aerobics throughout pregnancy did so at a relatively high overall level of performance and that, within this populace, pulse rate was a relatively poor predictor of exercise intensity. These women's level of exercise performance far exceeds that reported in earlier studies.[1, 2, 4-8] The fact that the hypothesized ill-effects were not observed at these relatively high levels of performance indicates that well-conditioned women probably can continue to exercise at relatively high intensities for periods of ≥30 minutes without detriment. Likewise, using a pulse rate of 140 beats/min to indicate a certain exercise intensity or level of effort in well-conditioned women during pregnancy appears imprecise.

In summary, these data, which were obtained in well-conditioned, low-risk women, indicate that, in this populace, continuing a regular exercise regimen of running or aerobics throughout pregnancy has a beneficial effect on multiple aspects of the labor process. Furthermore, despite a high overall level of performance, the anticipated complications were not observed. While several well-controlled studies suggest that these findings probably are not applicable to other types of women or exercise regimens,[1,2,4-8] they are reassuring and, in conjunction with the other published human data, suggest that current guidelines for exercise in pregnancy should be carefully evaluated to determine if the potential maternal benefits of exercise may be realized without increasing fetal and neonatal risk.

REFERENCES

1. Kulpa PJ, White BM, Visscher R. Aerobic exercise in pregnancy. Am J Obstet Gynecol 1987;156:1395-403.
2. Wong SC, McKenzie DC. Cardiorespiratory fitness and its effects on outcome. Int J Sports Med 1987;8:79-83.
3. Clapp JF, Dickstein S. Endurance exercise and pregnancy outcome. Med Sci Sports Exerc 1984;16:556-62.
4. Collings CA, Curet LB, Mullin JP. Maternal and fetal re-

sponses to a maternal aerobic exercise program. AM J OBSTET GYNECOL 1983;145:702-7.
5. Hall DC, Kaufman DA. Effects of aerobic and strength conditioning on pregnancy outcomes. AM J OBSTET GYNECOL 1987;157:1199-203.
6. Webb KA, Wolfe LA, Trammer JE, McGrath MJ. Pregnancy outcome following physical fitness training. Can J Sport Sci 1988;13:93-94P.
7. Erdelyi GJ. Gynecological survey of female athletes. J Sports Med Phys Fitness 1962;2:174-9.
8. Dale E, Mullinax KM, Bryan DH. Exercise during pregnancy: effects on the fetus. Can J Appl Sport Sci 1982;7:98-103.
9. Clapp JF. The effects of maternal exercise on early pregnancy outcome. AM J OBSTET GYNECOL 1989;161:1453-7.
10. Pollock ML, Wilmore JH, Fox SM. Exercise in health and disease. Philadelphia: WB Saunders, 1984:431.
11. Clapp JF, Capeless EL. Neonatal morphometrics after endurance exercise during pregnancy. AM J OBSTET GYNECOL 1990;163:1805-11.

Neonatal morphometrics after endurance exercise during pregnancy

James F. Clapp III, MD, and Eleanor L. Capeless, MD
Burlington, Vermont

This study was designed to test the hypothesis that continuation of a regular running and/or aerobics program during late pregnancy at or above 50% of preconceptional levels limits fetal growth. Accordingly, detailed neonatal morphometric data were gathered in the offspring of two groups: 77 well-conditioned recreational runners and aerobic dancers who were delivered at term after continuing their exercise regimen at or above 50% of the preconceptional level throughout pregnancy and 55 matched controls. Daily exercise performance was quantitated before conception and throughout pregnancy. Significant reductions in birth weight (−310 gm), birth weight percentile (−20), ponderal index (−0.24), its percentile (−30), and the fetoplacental weight ratio (−0.7) were seen in the offspring of the exercise group whereas crown-heel length (51.4 cm) and head circumference (35.0) were similar in the two groups. Reductions in two-site skin-fold thickness (−1.5 mm), skin-fold percentile (−30), calculated percent body fat (−5.0%), and fat mass (−220 gm) in the offspring of the exercise group confirmed the asymmetric pattern of growth restriction and indicated that approximately 70% of the difference in birth weight could be explained by the difference in neonatal fat mass. In runners, the relative level of exercise performance in the last 5 months of pregnancy explained 40% of the variability in birth weight over an 1100 gm birth weight range. We conclude that continuation of a regular aerobic or running program at or above a minimal training level during late pregnancy results in an asymmetric pattern of growth restriction that primarily impacts on neonatal fat mass. (AM J OBSTET GYNECOL 1990;163:1805-11.)

Key words: Exercise, pregnancy, birth weight

The interaction between exercise performance during pregnancy and pregnancy outcome is complex but appears to be based on the interplay between the maternal physiologic adaptations to exercise and the physiologic adaptations to pregnancy.[1-3] The magnitude of the exercise-induced adaptations is determined by the type, intensity, frequency, and duration of the exercise while the magnitude of the adaptations to pregnancy is a function of the time point in the pregnancy when the exercise is carried out. Given this number of variables, it is not surprising that the reported impact of exercise during pregnancy on birth weight has varied, with investigators reporting a reduction,[1,4] no effect,[5-8] or an increase.[9,10]

In an attempt to control the multiple variables involved, the current study was limited to well-conditioned recreational athletes who performed one of two forms of high-intensity exercise (running and aerobics) in which a large fraction of the body's muscle mass is used against gravity for relatively prolonged

From the Department of Obstetrics and Gynecology, The University of Vermont College of Medicine.
Supported in part by National Institutes of Health grant HD21268 and by grant 6-464 from the March of Dimes Birth Defects Foundation.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: James F. Clapp III, MD, Physiology Laboratory, Department of Obstetrics and Gynecology, Metro Health Medical Center, 3395 Scranton Road, Cleveland, OH 44109.
6/6/24722

Table I. Preconceptional fitness parameters in the two groups

Parameter	*Control*	*Exercise*	*Range*
AM pulse (beats/min)	55 ± 3	54 ± 3	48-62
Body fat (%)	17.2 ± 5.3	16.8 ± 4.9	10.2-26.6
$V\dot{O}_2$ max (ml/kg/min)	52.3 ± 6.8	53.8 ± 7.1	38.4-68.2

Data presented as mean ± SD.

periods of time. The experimental design included a carefully matched control populace, and individual exercise performance was prospectively monitored before conception and throughout pregnancy. The hypothesis tested was that when recreational running and/or aerobics are continued during late pregnancy, at or above 50% of preconceptional levels, they limit fetal growth. The objective outcome parameter was neonatal morphometry.

Material and methods

Between July 1983 and January 1990, we prospectively monitored the spontaneous daily exercise performance of 42 recreational runners and 35 aerobic dancers who exercised for at least 30 minutes, three or more times each week before conception and who were delivered at term (>266 and <294 days) after continuing their chosen form of exercise at or above 50% of the preconceptional level throughout pregnancy. These women constituted the exercise group. Exercise performance was quantitated before and during pregnancy by measuring heart rate and the duration of exercise daily during field exercise. These data, coupled with the periodic laboratory evaluation, were used to quantitate weekly and monthly exercise performance by means of the training effect method and were expressed as a duration-intensity index (product of weekly duration and average weekly intensity).[11]

We also enrolled and monitored a control group of 55 physically active, fit women who did not plan to maintain a continuous, high-intensity exercise regimen after confirmation of pregnancy (first trimester). They were matched with the exercise group for general health, physical fitness, education, family income, age, parity, contraceptive use, pregravid weight, job type, dietary intake, sleep-activity cycles, smoking, and alcohol intake. Before pregnancy these women either ran, performed aerobics, or played racquet sports on a more intermittent basis than those in the exercise group and their exercise performance was monitored in a similar fashion.

All pregnancies included in the analysis were clinically normal, each subject had between 11 and 14 antenatal visits with a private obstetrician, and all were delivered at term. An additional 33 subjects who were initially enrolled in the project before conception and who were excluded from this analysis included 22 cases of either infertility or abortion (9 control, 13 exercise), three cases of mild pregnancy-induced hypertension (two control, one exercise), and seven cases in which labor began before 266 days' gestation (four exercise, three control). Written informed consent was obtained from each subject before enrollment. Pregnancies were confirmed within a week of missed menses (by β-subunit human chorionic gonadotropin) and the dates were confirmed by ultrasonographic examination at 53 to 60 days after the last menstrual period. The relationship between exercise intensity and heart rate was determined before conception and every 6 to 8 weeks during pregnancy by measuring heart rate and oxygen consumption during either treadmill exercise or aerobics. A crude estimate of historic dietary intake (kilocalories and component mix) was obtained by questionnaire at the time of initial preconceptional evaluation. At each testing session during pregnancy, all subjects were questioned to determine if there had been a significant change in the quantity or quality of their diet.

Neonatal and placental morphometric measures were obtained within 2 hours of birth by one of two individuals. After the infant had been carefully dried, weight was determined by the nearest 10 gm with an accurately calibrated balance beam or electronic scale. The measurement of crown-heel length to the nearest millimeter was carefully standardized by use of a specially constructed measurement box and the tonic neck reflex.[12] This technique had a coefficient of variation of <1% and, on the basis of a comparison with the values charted by the nurses, resulted in a shorter (2% to 5%) length than that obtained with the routine clinical method. Circumferential measurements of the head, chest, and abdomen were obtained to the nearest millimeter with a narrow flexible tape used in the recommended fashion.[13] The coefficient of variation of each measurement was <3%. Triceps and subscapular skinfold thicknesses were measured on the right side with John Bull calipers and had coefficients of variation under 6%. Ponderal index was calculated as birth weight in grams × 100 divided by the cube of the crown-heel length in centimeters.

These morphometric data were referenced to normative values for birth weight, crown-heel length, ponderal index, circumferences, and skin-fold thicknesses established in a random sample (n = 335) of our overall term newborn populace to determine percentiles. (These normative data are being submitted separately for publication and therefore are not detailed here.) The neonatal sample was obtained over a 2½-year period by use of random grab sampling throughout. With birth weight used as the objective criterion, the sample

Table II. Additional maternal characteristics

Parameter	*Control*	*Exercise*	*Significance*
Age (yr)	30.1 ± 3.3	30.6 ± 2.5	NS
Pregravid weight (kg)	58.1 ± 5.9	57.7 ± 5.2	NS
Weight gain (kg)	16.4 ± 4.2	13.5 ± 4.2	0.01
Parity	0.63 ± 0.75	0.59 ± 0.59	NS
Gestational age (days)	281 ± 6	279 ± 6	NS
Smoking (No. of cigarettes/day)	0	0	NS
Alcohol intake (No. of alcoholic drinks/wk)	0.32 ± 0.45	0.29 ± 0.38	NS
Hematocrit before pregnancy (%)	42.3 ± 1.6	41.9 ± 1.8	NS
Hematocrit at 36 wk (%)	38.2 ± 1.9	37.6 ± 1.5	NS
Blood glucose (mmol/L)	4.88 ± 0.61	4.83 ± 0.70	NS

Data presented as mean ± SD. Alcohol intake is the average number of mixed drinks, glasses of wine, or cans of beer each week during pregnancy. Blood glucose was measured on whole blood at approximately 36 weeks' gestation on a random postprandial sample obtained at standing rest.

was normally distributed and statistically no different from that of all term infants born at the institution. The skin-fold percentile was used to estimate body fat, which had been determined with the original Dauncy method and formula in the reference populace.[14] Within that populace the r value between skin-fold thicknesses and calculated percent body fat was 0.95.

After gross inspection and removal of surface clot, cord, and membranes, each placenta was blotted and weighed to the nearest 5 gm. Its volume was determined to the nearest 5 ml by saline solution displacement and its chorionic surface area was measured to the nearest square centimeter by planimetry.[15]

Significant between-group differences for normally distributed parameters were detected with the unpaired Student's t test. Other parameters were assessed with the Wilcoxon–Mann-Whitney test. Single and multiple regression analysis was used to seek a dose-response relationship between exercise performance and morphometric outcome. Significance was set at the 0.05 level.

Results

Maternal characteristics. In both groups the subjects were all from the middle or upper socioeconomic class and worked part-time or full-time. All ate a well-balanced, high-complex-carbohydrate diet with adequate calories and had similar rest-activity patterns before conception. As shown in Table I, before conception all women in both groups were fit as assessed by morning pulse rate, maximal oxygen consumption, and percent body fat. There were no significant between-group differences in these parameters before conception. In this study populace these specific measurements were not routinely repeated during pregnancy.

Several additional maternal obstetric and demographic characteristics known to influence birth weight are listed in Table II. Data on maternal hematocrit and random postprandial resting blood glucose level in late pregnancy are also included. The only significant between-group difference was pregnancy weight gain, which was 2.9 kg less in the exercise group. As the women who performed aerobics experienced only a minimal decrease in weight gain (overall weight gain 15.3 ± 4.1 kg), the difference was mainly due to a decrease in pregnancy weight gain in the runners (overall weight gain 12.1 ± 3.7 kg). On the basis of subjective recall data, these differences in weight gain did not appear to be due to a different pattern of change in dietary intake or in the amount of dietary intake at any point in time in the two groups, which were roughly equivalent over the course of pregnancy.

Maternal exercise performance. Before conception, running performance ranged between 16 and 68 km each week at a pace between 4.3 and 6 min/km and an intensity ranging between 51% and 83% of maximum. The average weekly duration-intensity index for the runners was 14,860 ± 353 (mean ± SD). During pregnancy, individual performance was quite variable but, as a group, overall running performance fell progressively until the sixth lunar month and then stabilized for the remainder of the pregnancy. Individual performance ranged between 50% and 109% of preconceptional levels for the entire pregnancy with an average of 69% ± 16%. In the last five lunar months it averaged 65% ± 18% of preconceptional levels with individual performances ranging between 50% and 122%.

Before pregnancy, participation in aerobics ranged between 3 and 11 sessions per week with a median of 4. Exercise intensity during warm-up and cool-down ranged between 20% and 40% of maximum and 54% to 90% of maximum capacity during the 25- to 30-minute so-called "aerobic" phase of the workout. The average duration-intensity index of 12,372 ± 311 was slightly less than that seen in the runners. During pregnancy the pattern of overall aerobic performance was similar to that seen with running, and individual variation was again the rule rather than the exception. In-

Table III. Effect of exercise on neonatal morphometrics

Parameter	*Control*	*Exercise*	*Significance*
Birth weight (gm)	3691 ± 348	3381 ± 322	0.01
Birth weight percentile	65 ± 19	45 ± 22	0.01
Length (cm)	51.3 ± 1.5	51.4 ± 1.3	NS
Ponderal index	2.71 ± 0.18	2.47 ± 0.21	0.01
Ponderal index percentile	63 ± 25	33 ± 22	0.01
Head circumference (cm)	35.2 ± 1.1	35.0 ± 1.0	NS
Placental weight (gm)	456 ± 83	449 ± 66	NS
Fetoplacental weight ratio	8.28 ± 1.34	7.61 ± 1.10	0.05

Data presented as mean ± SD. Length = crown-heel length.

Table IV. Effect of exercise type on neonatal morphometrics

Parameter	*Running*	*Aerobics*	*Significance*
Birth weight (gm)	3301 ± 267	3481 ± 357	NS
Birth weight percentile	43 ± 20	48 ± 24	NS
Length (cm)	51.1 ± 1.4	51.7 ± 1.1	NS
Ponderal index	2.46 ± 0.19	2.51 ± 0.24	NS
Ponderal index percentile	31 ± 22	36 ± 23	NS
Head circumference (cm)	34.8 ± 0.8	35.3 ± 1.1	NS
Placental weight (gm)	461 ± 68	431 ± 63	NS
Fetoplacental weight ratio	7.16 ± 0.87	8.16 ± 1.06	0.01

Data presented as mean ± SD. Length = crown-heel length.

dividual performance ranged between 50% and 98% of preconceptional levels for the entire pregnancy with a group average of 67% ± 13%. In the last five lunar months it averaged 64% ± 13% of preconceptional values with individual performance ranging between 50% and 92%.

Before pregnancy the control women ran, performed aerobics, or participated in racquet sports intermittently at a frequency that ranged between two and eight times per month. The preconceptional duration-intensity index averaged 2936 ± 284 with the highest individual value being 6440. During pregnancy the majority stopped exercise completely before or shortly after confirmation of viability (n = 37), while the remainder continued to play racquet sports infrequently (once a week or less). In the latter, the weekly duration-intensity index ranged between 0 and 1550 after the end of the first trimester, with only three subjects consistently exceeding a weekly duration-intensity index of 1000 for >1 week each month.

Neonatal morphometrics. These data are detailed by group and subgroup in Tables III to V. Note that, whereas length, head circumference and placental weight were similar in the two groups, birth weight, birth weight percentile, ponderal index, its percentile, and the fetoplacental weight ratio all were decreased in the offspring of the women who continued to exercise throughout pregnancy. These differences are consistent with a pattern of brain-sparing, soft-tissue growth restriction, or so-called late-onset intrauterine growth restriction,[16] and appear more marked in the offspring of the runners than in the offspring of those who performed aerobics (Table IV).

The higher head circumference/abdominal circumference ratio and the lesser skin-fold thickness in the offspring of the exercising women (Table V) also are consistent with this pattern of intrauterine growth restriction and suggest that a large fraction of the difference in birth weight may simply be a reduction in fat mass in the offspring of the exercising women. Linear regression of estimated fat mass and birth weight supports this suggestion. There is a strong, direct relationship between fat mass (calculated from skin folds) and birth weight in the entire populace (r = 0.8050), such that the weight of neonatal fat explains approximately 65% of the variability in birth weight over a birth weight range between 2780 and 4120 gm. Indeed, differences in calculated body fat explain >70% of the difference in birth weight between the control infants and those of the women who continued to exercise throughout pregnancy. In the aerobics subgroup it explains 99% of the difference and in the running subgroup 60% (Table V).

Neonatal course. No infants in either group had an abnormal Dextrostix screening and, despite the differences in fat mass, only one infant in each group had a rectal temperature <36.0° C in the immediate neonatal period. Hematocrits were not routinely obtained and all infants left the hospital within 72 hours of birth.

Correlation between exercise performance and birth weight. As detailed in Table VI, there was no correlation between exercise performance before con-

Table V. Indices of soft tissue growth restriction

Parameter	*Control*	*Exercise*	*Significance*
Head circumference/abdominal circumference ratio	1.06 ± 0.05	1.13 ± 0.05	0.05
Skin folds* (mm)	11.8 ± 2.1	9.3 ± 1.6	0.01
Skin-fold percentile	58 ± 25	28 ± 20	0.01
Calculated body fat (%)	16.2 ± 4.9	11.2 ± 3.1	0.01
Calculated fat mass (gm)	603 ± 231	382 ± 136	0.01
Calculated lean mass (mean) (gm)	3088	3000	

Data presented as mean ± SD.
*Sum of triceps and subscapular thickness.

ception and birth weight. The same was true for the absolute amount of exercise performed during any portion or all of pregnancy in the women who continued to exercise regularly, with the highest R^2 being 0.0032. However, when exercise performance during pregnancy was expressed as a percentage of that performed before conception, there was a significant but weak linear correlation between the overall relative level of exercise performance during pregnancy and birth weight. The correlation improved when it was corrected for the stage of pregnancy in which the exercise was performed and exercise type, indicating that exercise in late pregnancy and running had the most significant quantitative effects. Thus the relative level of exercise performance in the latter half of pregnancy explained approximately 40% of the variance in the birth weight of runner's offspring over an 1100 gm birth weight range. Additional least-squares analysis with the use of a power function fit improved this correlation slightly ($R^2 = 0.4437$). In contrast, although the offspring in the aerobics group were >200 gm lighter than the control infants, neither the absolute level nor the relative level of aerobic exercise performance during pregnancy correlated with the birth weight of the offspring.

Additional correlates with birth weight. Multiple potential confounding variables were also assessed for a possible association with birth weight. Significant correlations included placental weight ($R^2 = 0.0654$) and pregnancy weight gain ($R^2 = 0.2677$) for the entire populace. In the exercise group these correlations improved to $R^2 = 0.1106$ and $R^2 = 0.4356$ for placental weight and pregnancy weight gain, respectively. Additional potential confounding variables either were uniform within the populace (gestational age, smoking, socioeconomic and nutritional status) or had an $R^2 < 0.03$ when examined individually (preconceptional weight, height, parity, and sex of offspring). Therefore multiple regression analysis for factors influencing birth weight was limited to three independent variables in the running subgroup: relative exercise performance for the last 5 months of pregnancy, placental weight, and pregnancy weight gain.

Table VI. Correlations between exercise and birth weight

Exercise variable	*Exercise type*	R^2
Overall performance PTP	Both or either type	0.0002
Overall performance TP	Both types	0.0675
Overall performance TP	Running	0.3567
Overall performance TP	Aerobics	0.0010
Performance early preg.	Both types	0.0494
Performance early preg.	Running	0.2174
Performance late preg.	Both types	0.1164
Performance late preg.	Running	0.4034
Performance late preg.	Aerobics	0.0027

Overall performance PTP, Average duration-intensity index before conception; *Overall performance TP*, average duration-intensity index during the total pregnancy divided by the average duration-intensity before conception; *Performance early preg.*, average duration-intensity index for the first five lunar months of pregnancy divided by the average duration-intensity before conception; *Performance late preg.*, average duration-intensity index for the last five lunar months of pregnancy divided by the average duration-intensity before conception.

Table VII. Fetoplacental weight ratios

Placental weight range (gm)	*Control ratio*	*Exercise ratio*
<400	9.75 ± 1.07	9.08 ± 0.74*
400-450	8.87 ± 0.88	7.76 ± 0.66*
451-500	7.54 ± 0.62	7.36 ± 0.43*
>501	7.32 ± 0.60	6.45 ± 0.44*

Data presented as mean ± SD.
*$p < 0.05$.

The addition of placental weight and pregnancy weight gain to the analysis explained only an additional 5% of the variance in birth weight ($R^2 = 0.4493$), suggesting that there were discrete associations between the three independent variables. The multiple correlation coefficients between birth weight and either placental weight combined with relative exercise performance or weight gain and relative exercise performance were similar (r values of 0.6341 and 0.6411, respectively), confirming that both placental weight and maternal weight gain, like birth weight, were independently related to running performance in the last 5

months of pregnancy. This was further substantiated by the correlations obtained between relative exercise performance over the latter half of pregnancy and both placental weight ($r = -0.3841$) and pregnancy weight gain ($r = -0.5881$). Thus in the running subgroup any birth weight effect of placental weight or pregnancy weight gain appeared to be due to the impact of running performance on each of them.

Exercise and the fetoplacental weight ratio. Although placental weight was similar in the two groups, the fetoplacental weight ratio was significantly decreased in the exercise group (Table II), suggesting that fetal growth per unit weight of placenta was restricted by exercise. To explore this possibility we calculated the fetoplacental weight ratio by placental weight in 50 gm increments. These data, shown in Table VII, demonstrate that the ratio is significantly decreased in the exercise group in all four weight groups. Linear regression of these data indicate that the slope of the relationship is similar in the two groups but that the intercept is reduced in the exercise group. As a result the average between-group difference in birth weight with placental weights between 300 and 600 gm averages 75 gm per 100 gm of placental weight.

Comment

At the outset it should be emphasized that these data were gathered from a select group of well-conditioned recreational athletes who had been performing two specific types of exercise on a regular basis for at least 2 years before conception and a similar select group of controls. Thus extrapolation of these findings to other groups of women and/or other exercise regimens is not warranted. With this limitation, these data strongly support the initial hypothesis, namely, that fetal growth is restricted when a regular running or aerobics program is maintained >50% of preconceptional levels during pregnancy. They also confirm the directional birth weight effect observed in earlier studies with runners and aerobic dancers in which an attempt was made to quantify exercise performance during pregnancy.[1, 4]

It is possible but unlikely that the differences in birth weight were due to differences in fitness between the two groups. First, while the control women spent much less time in exercise before conception, their level of fitness, as assessed by body composition, AM pulse rate, and maximum oxygen consumption, was not significantly different from that of the women who constituted the exercise group. Second, several studies that have assessed the effects of an exercise regimen on both fitness and birth weight have noted that fitness had either no effect or a positive effect on birth weight.[5, 8, 10] Third, as discussed in an earlier publication, others have noted a positive correlation between one measure of fitness during pregnancy, work capacity, and birth weight.[1] Finally, although not actually measured in the present study, the women who continued to exercise throughout pregnancy were probably more fit at the time of delivery and, if fitness were the important variable, they should have had heavier, not lighter, infants as compared with the control populace.

As discussed elsewhere,[16] the effect of caloric quality and quantity on birth weight is well known, but in the human, even with heavy physical activity, it appears to have a restrictive effect only when caloric intake is severely curtailed, and this is always associated with poor maternal weight gain.[17, 18] As the subjects in both groups experienced adequate weight gain (control, 16.4 kg; exercise, 13.5 kg) and by history followed diets of comparable quality, it is unlikely that dietary intake per se had a direct effect on the morphometric outcome.

The additional morphometric parameters indicate that the growth restriction appears limited to soft tissue and for the most part appears to be limited to a restriction of fat deposition, which is reassuring from a public health point of view. Further studies of body composition in similar infants with the use of more sophisticated technology (total body electrical conductivity) are planned to confirm these findings as are long term follow-up studies to determine if the decrease in fat mass at birth is maintained later in life. If so, this effect of regular exercise in pregnancy may have preventive value in terms of the risk of obesity in later life.

The data also demonstrate that different types of exercise and the time in pregnancy when the exercise is performed have different effects on neonatal morphometry and perhaps on other indices of pregnancy outcome. This is entirely consistent with the variable effects of exercise on various facets of pregnancy outcome reported with different exercise regimens to date,[1, 4-11] and indicates that each type of exercise and exercise regimen needs to be separately evaluated. The fact that a dose-response effect could be demonstrated with running and not with aerobics is a good example of this and requires clarification. However, the data obtained to date suggest that the two types of exercise may evoke different overall maternal physiological responses that may be related to the limited duration and variable intensity of aerobics versus running.[19] The observed reduction in neonatal fat mass with both forms of exercise is entirely consistent with a late pregnancy effect. Additional studies with exercise limited to either early or late pregnancy are needed to confirm this time-specific effect.

The finding of a consistent decrease in the fetoplacental weight ratio over more than a 250 gm placental weight range is of interest as it is quite different from that seen in a variety of animal models used to study fetal growth restriction.[16] In the animal model the ratio

is unchanged with mild and moderate degrees of restriction and increases with extreme growth restriction. Thus the decreased ratio associated with endurance exercise in the human may be indicative of a unique protective placental adaptation to the stimulus of recurrent fluctuations in uterine blood flow and/or substrate delivery. In any case it indicates that further study is warranted and must be conducted in the human.

In summary, the data obtained in this serial study of a relatively uniform, well-conditioned group of women support the initial hypothesis. In this populace they indicate that continuing aerobic and/or running regimens at or above a minimum training level in the latter half of pregnancy has a defined impact on fetal growth that is evidenced at birth by a reduction in birth weight that is primarily due to a reduction in neonatal fat mass. They also confirm that the interaction between the physiologic adaptations to exercise and the physiologic adaptations to pregnancy are multifaceted and warrant further study.

REFERENCES

1. Clapp JF, Dickstein S. Endurance exercise and pregnancy outcome. Med Sci Sorts Exerc 1984;16:556-62.
2. Clapp JF, Wesley M, Sleamaker RH. Thermoregulatory and metabolic responses to jogging prior to and during pregnancy. Med Sci Sports Exerc 1987;19:124-30.
3. Clapp JF, Seaward BL, Sleamaker RH, Hiser J. Maternal physiological adaptations to early human pregnancy. AM J OBSTET GYNECOL 1988;159:1456-60.
4. Dale E, Mullinax KM, Bryan DH. Exercise during pregnancy—effects on the fetus. Can J Appl Sports Sci 1982;7:98-102.
5. Collings CA, Curet LB, Mullin JP. Maternal and fetal responses to a maternal exercise program. AM J OBSTET GYNECOL 1983;145:702-7.
6. Jones RL, Botti JJ, Anderson WM, Bennett NL. Thermoregulation during aerobic exercise in pregnancy. Obstet Gynecol 1985;65:340-5.
7. Sibley L, Ruhling RO, Cameron-Foster J, Christensen C, Bolen T. Swimming and physical fitness during pregnancy. J Nurse Midwif 1981;26:3-12.
8. Kulpa PJ, White BM, Visscher R. Aerobic exercise during pregnancy. AM J OBSTET GYNECOL 1987;156:1395-403.
9. Jarrett JC, Spellacy WM. Jogging during pregnancy: an improved outcome. Obstet Gynecol 1983;61:705-9.
10. Hall DC, Kaufmann DA. Effects of aerobic and strength conditioning on pregnancy outcomes. AM J OBSTET GYNECOL 1987;157:1199-203.
11. Clapp JF. The effects of maternal exercise on early pregnancy outcome. AM J OBSTET GYNECOL 1989;161:1453-7.
12. Miller HC, Hassanein K. Diagnosis of impaired fetal growth in newborn infants. Pediatrics 1971;48:511-22.
13. Seefeldt VD, Harrison GG. Infants, children and youth. In: Lohman TG, Roche AF, Martorell R, eds. Anthropometric standardization reference manual. Champaign, Illinois: Human kinetics. 1988:111-4.
14. Dauncey MJ, Gandy G, Gairdner D. Assessment of total body fat in infancy from skinfold measurements. Arch Dis Child 1977;52:223-7.
15. Adair FL, Thelander H. A study of the weight and dimensions of the human placenta and its relation to the weight of the newborn infant. AM J OBSTET GYNECOL 1925;10:172-94.
16. Clapp JF. Uteroplacental blood flow and fetal growth. In: Sharp F, Milner RDG, Fraser RB, eds. Fetal growth. London: The Royal College of Obstetricians and Gynaecologists, 1989:235-44.
17. Tafari N, Naeye RL, Gobezie A. Effects of maternal undernutrition and heavy physical work during pregnancy on birthweight. Br J Obstet Gynaecol 1980;87:222-6.
18. Briend A. Maternal physical activity, birth weight and perinatal mortality. Med Hypoth 1980;6:1157-70.
19. Clapp JF. The effects of exercise on uteroplacental blood flow. In: Rosenfeld CR, ed. The uterine circulation. Ithaca, New York: Perinatology Press, 1989:299-310.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Does fasting interval affect the glucose challenge test?

Michael D. Berkus, MD,[a] Michael P. Stern, MD,[b] Braxton D. Mitchell, PhD,[b] Edward R. Newton, MD,[a] and Oded Langer, MD[a]

San Antonio, Texas

The relationship between fasting interval and glucose screening was assessed in a prospective study of 153 non-diabetic pregnant patients undergoing a standard 50g glucose challenge test. An interval of less than 3 hours after the last meal was found to be associated with a significantly greater insulin response as opposed to a fasting interval of more than 3 hours (121.3 pmol/l vs. 83.5 pmol/l, $p < .001$) and a greater insulin/glucose index (0.92 vs. 0.66, $p < .001$), with no difference in plasma glucose. Overall, obese patients had a higher glucose response than non-obese, but only higher insulin levels (107 pmol/l vs. 69 pmol/l, $p < .001$) and insulin/glucose index (0.79 vs. 0.59, $p < .02$) when the fasting interval was greater than 3 hours. Our data suggest that the fasting interval can influence insulin response during a glucose challenge test and the aberration in insulin secretion may effect screening results, especially in the high-risk gravida with glucose abnormality. (Am J Obstet Gynecol 1990;163:1812-7.)

Key words: Gestational diabetes, glucose screening, fasting interval, insulin, obesity

The American College of Obstetrics and Gynecologists Technical Bulletin recommended that a 50 gm oral glucose screening should be performed on all pregnant women 30 years of age or older, and on any women with risk factors such as a family history of diabetes or obesity. This screening should be performed between 24 and 28 weeks of gestation "without regard to time of last meal or time of day" and if the plasma glucose level measured 1 hour after the 50 gm load exceeds 140 mg/dl, a 3-hour oral glucose tolerance test should be administered.[1] Although extensive research has been performed regarding methods of glucose screening to determine the best threshold for identifying patients at risk for gestational diabetes,[2-4] a paucity of information exists as to the influence of a fasting or fed state on screening values,[5] and no study has examined the influence of fasting interval on insulin response to a glucose challenge test.

Previously, in the report of Coustan et al.,[5] it was found that the glucose response was significantly higher if patients with gestational diabetes had fasted than if they had been given a standard 600 kcal meal 1 hour before testing. They had hypothesized that the screening test value was lower in the fed state because previous ingestion of a mixed meal dampened the usual state of insulin resistance observed in fasting pregnant women.[5] They did not measure insulin levels in that study to confirm their hypothesis. The screening of pregnant patients after a standard meal may not be applicable to tests administered after the ingestion of unknown foodstuffs. Additionally, a 1-hour time interval may not be representative of other time intervals. Of significance is that examination of nonpregnant women with type II diabetes has shown an effect of protein loading on plasma glucose response to a 50 gm screening test after 4 hours and no significant effect at 1 hour. Also, the insulin response after a mixed loading was much greater than after a 50 gm glucose challenge alone.[6] Finally, Cheney et al.[7] have shown that obese women with gestational diabetes manifest higher glucose and insulin values after a 400 kcal breakfast meal, as compared with nonobese patients with gestational diabetes.

Thus it seems that pregnant patients with gestational diabetes respond to a glucose challenge test differently whether they are in the fasting or fed state and that this response is further modified by maternal size. However, it is not clear in subjects with an elevated screening result but a normal oral glucose tolerance test result to what extend insulin resistance plays a role in insulin response. Furthermore, there is no information regarding the relationship of fasting interval and obesity with glucose and insulin secretion when only the glucose challenge test result is abnormal.

Since the recommendation is to screen patients with a glucose challenge test without regard to timing or content of the last caloric intake, we chose to study mothers under this guideline[1] to discern the impact on screening results. Thus the purpose of this study was to examine the effect of fasting interval on glucose and insulin response to a glucose challenge test, to ascertain the influence of obesity and fasting interval on screen-

From the Department of Obstetrics and Gynecology[a] and the Department of Medicine,[b] The University of Texas Health Science Center at San Antonio.

Supported in part by National Institutes of Health grant HL-24799.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St Louis, Missouri, March 21-24, 1990.

Reprint requests: Michael D. Berkus, MD, Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836.

6/6/24719

Table I. Analysis of variance of hourly fasting intervals

Fasting interval (hr)	*n*	*Glucose (mg/dl)*	*Insulin (pmol/L)*	*Insulin/glucose index*
0-1	20	118 ± 28	129 ± 82*	1.04 ± 0.6†
1-2	24	123 ± 24	114 ± 65*	0.89 ± 0.3†
2-3	25	120 ± 27	111 ± 91*	0.85 ± 0.5†
>3	84	120 ± 23	80 ± 51	0.65 ± 0.4
		(F = 0.168)	(F = 4.28)	(F = 5.47)

*0 to 1 hour, 1 to 2 hours, or 2 to 3 hours versus > 3 hours, $p < 0.04$.
†0 to 1 hour, 1 to 2 hours, or 2 to 3 hours versus > 3 hours, $p < 0.05$.

ing results, and to determine whether alterations in glucose and insulin secretion seen in women with gestational diabetes are present with minor degrees of glucose abnormality.

Material and methods

Nonhypertensive pregnant women were recruited from the obstetrics clinic of The University of Texas Health Science Center at San Antonio (UTHSCSA) and from other San Antonio–area clinics. The subjects were between 24 and 28 weeks of gestation or late registrants after 28 weeks and were not receiving insulin at the time of the study. The subjects included presumed normal pregnant women about to undergo a routine 50 gm glucose screening for gestational diabetes. Patients were enrolled without regard to their fasting state on an availability basis. The study was approved by the Institutional Review Board of the UTHSCSA and all subjects gave informed consent.

Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. For the purpose of analysis patients were considered to be obese if their body mass index was ≥27. Patients' glucose screening results were considered to be normal if the values were <140 mg/dl, to be abnormal if the values were ≥140 mg/dl, and to have gestational diabetes when two values on the oral glucose tolerance test were abnormal. Those mothers with gestational diabetes were excluded from the analysis. The patients were asked the number of hours since they had last eaten or drunk liquids other than water to determine the fasting time–to–screening interval. This value was then used as the fasting interval for the study. All testing was completed during morning clinics.

Blood samples were drawn 1 hour after a 50 gm load of a glucose equivalent (Koladex or Orangedex, Custom Laboratories, Baltimore) was administered. Plasma glucose level was determined with the use of the glucose oxidase method measured with a Paramax chemistry analyzer (American Dade Chemical Systems, Irvine, Calif.). Serum insulin concentrations were measured with a commercial solid-phase radioimmunoassay procedure (Diagnostic Products, Los Angeles). The coefficient of variation between duplicate aliquots measured at the same time was 8.1% for the 1-hour concentrations of insulin.[8] The insulin/glucose index was calculated to adjust for differing glucose levels.

Data analysis included bivariate and multivariate statistical techniques with parametric and nonparametric tests. Categoric data were analyzed with χ^2 and Fisher's exact tests. Continuous data were analyzed by Student's t test. One-way analysis of variance was used to compare different fasting intervals.

Results

A total of 163 patients participated in this prospective study. There were 119 women with a normal glucose screen (<140 mg/dl) and 44 with a glucose value ≥140 mg/dl. Of the 45 subjects with an abnormal screen, 10 had two abnormal values on the oral glucose tolerance test and were excluded from the analysis; this resulted in a total of 153 patients for evaluation.

Examination of fasting intervals of all subjects by means of analysis of variance for hourly groupings yielded significant differences for insulin and insulin/glucose index levels but not for plasma glucose values (F = 4.28, 5.47, and 0.16, respectively) (Table I). Subsequent post hoc t tests between hourly mean insulin response values yielded significantly greater values between 0 and 1 hour, 1 and 2 hours, and 2 and 3 hours and intervals >3 hours but no differences between intervals of 0 to 1 hour, 1 to 2 hours, or 2 to 3 hours. Therefore for the purpose of analysis the patients were stratified into two groups by a fasting interval of 0 to 3 hours, representing the "fed" patients, and ≥3 hours, representing "fasting" patients.

As shown in Table II, there were no significant demographic differences between groups, i.e., the "fed" group that ate within 3 hours of the test and the "fasting" group that had eaten 3 to 20 hours before being screened. All the patients were from the routine obstetrics clinic and were of similar age, parity, racial composition, gestational age, incidence of obesity, incidence of abnormal screening results, and plasma glucose level. Notably, both insulin level and the insulin/glucose index were significantly elevated in the "fed" group (<3 hours). The differences in insulin response were due

Table II. Patient characteristics and effect of 3-hour fasting interval

	"Fed" (mean = 1.6 ± 0.8 hr) (n = 69)	*"Fasting" (mean = 10.9 ± 5.0 hr) (n = 84)*	*p Value*
Age (yr)	25.0 ± 6	23.6 ± 5	NS
Primiparous (%)	6.5	3.9	NS
Multiparous (%)	38.6	51	NS
Hispanic (%)	32.7	39.9	NS
Gestational age (wk)	30.2 ± 5	29.6 ± 6	NS
Obesity (No.)	26 (37.7%)	29 (34.9%)	NS
Abnormal screen (No.)	18 (26.1%)	16 (19.0%)	NS
Glucose (mg/dl)	120 ± 26	120 ± 23	NS
Insulin (pmol/L)	117 ± 79	84 ± 63	0.005
Insulin/glucose index	0.92 ± 0.5	0.70 ± 0.5	0.009

Table III. Effect of 3-hour fasting interval on insulin response

	n	*"Fed" (< 3 hr)*	*"Fasting" (> 3 hr)*	*p Value*
Insulin (pmol/L)				
Normal (glucose < 140 mg/dl)	119	88 ± 43	82 ± 66	NS
Abnormal (glucose ≥ 140 mg/dl)	34	198 ± 101	94 ± 44	0.0006
Insulin/glucose index				
Normal (glucose < 140 mg/dl)	119	0.8 ± 0.4	0.7 ± 0.4	NS
Abnormal (glucose ≥ 140 mg/dl)	34	1.26 ± 0.6	0.6 ± 0.3	0.0004

entirely to patients with abnormal glucose screens (≥140 mg/dl) (Table III). As in insulin response the values for the corrected index (insulin/glucose) are significantly greater only for women with an abnormal screening.

Since obesity has been shown to have an influence on insulin resistance, the additional effect of obesity on glucose and insulin response was analyzed. The glucose response was higher for obese patients when the fasting interval was >3 hours (Fig. 1). Dividing glucose response for normal and abnormal glucose groups yielded significantly elevated values only for the normal "fasting" obese group. The insulin response in obese patients was greater than in nonobese women but only for the "fasting" group (Fig. 2). This was true whether the glucose screening was normal (<140 mg/dl) or abnormal. Also, for nonobese or lean patients in the abnormal group, the insulin response was significantly more for "fed" lean than "fasting" lean mothers, despite the small number of patients in these categories. When the insulin/glucose index or ratio was examined, the significance pattern was similar; however, the difference in the abnormal "fasting" group (glucose ≥140 mg/dl) was only marginally significant for obese versus lean (0.73 ± 0.3 vs 0.53 ± 0.2, $p = 0.09$) (Fig. 3).

Comment

A screening test should reliably detect individuals at risk for a disease in a safe, inexpensive, and convenient manner. The 50 gm oral glucose challenge test has been shown to meet these criteria.[2] Giving the test without regard to time of last meal adds to the convenience of the test and allows for more rapid assessment when necessary in advanced gestation. However, no previous studies have attempted to determine the effect, during glucose screening, of a variable time interval from the last meal on glucose and insulin results. This is the first report of the insulin response to a glucose challenge under the recommendation of The American College of Obstetricians and Gynecologists.[1]

The key findings in this study support the concept that the fasting interval can influence insulin response during a glucose challenge test and that maternal size may further modulate this effect. A fasting interval of up to 3 hours was found to be the critical interval in this study. Stratifying the data according to these results (<3 hours and ≥3 hours) yielded significant differences in insulin but not in glucose response. This difference was present only in the abnormal screening group, i.e., plasma glucose level ≥140 mg/dl. Obese subjects did have higher plasma glucose values than nonobese women when "fasting" and also had a greater insulin response than lean patients when the fasting interval was ≥3 hours. When the glucose screen was abnormal, both obese and nonobese gravid women had decreased insulin response ≥3 hours or more after eating. When the screen was normal (glucose <140 mg/dl), only lean subjects had a decreased response when "fasting."

Thus fasting interval did affect insulin response and resistance as reflected by the insulin/glucose index, and obesity further modified the results. The final question addressed by the study was to determine whether the

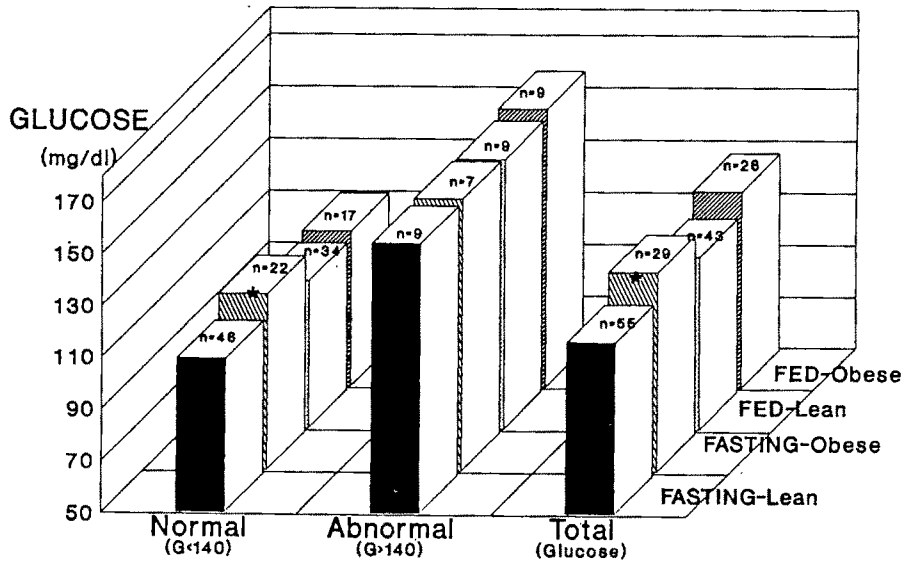


Fig. 1. Effect of fasting interval and obesity on glucose response. *FED-Obese,* Fasting interval <3 hours and body mass index ≥27. *FED-Lean,* Fasting interval <3 hours and body mass index <27. *FASTING-Obese,* Fasting interval ≥3 hours and body mass index ≥27. *FASTING-Lean,* Fasting interval ≥3 hours and body mass index <27.

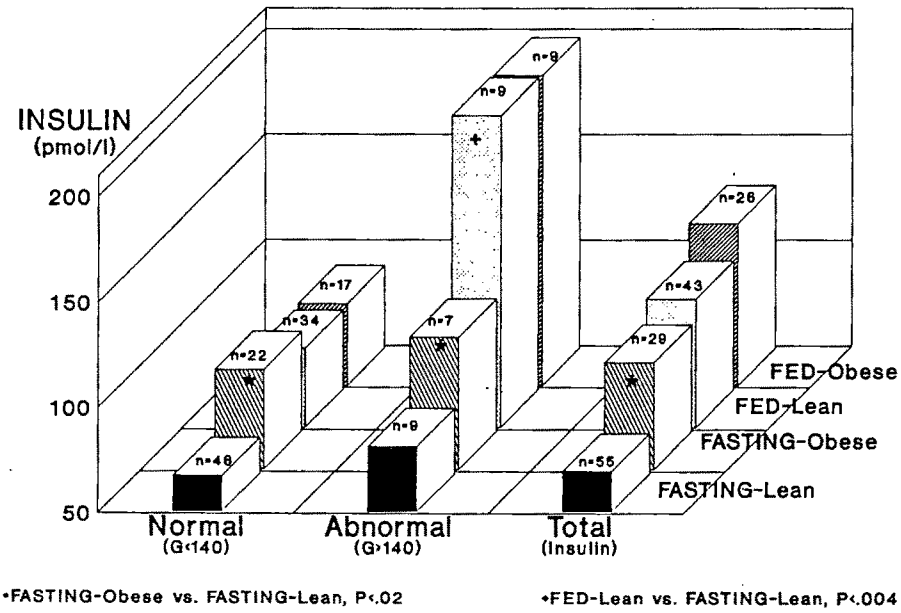


Fig. 2. Effect of fasting interval and obesity on insulin response. *FED-Obese,* Fasting interval <3 hours and body mass index ≥27. *FED-Lean,* Fasting interval <3 hours and body mass index <27. *FASTING-Obese,* Fasting interval ≥3 hours and body mass index ≥27. *FASTING-Lean,* Fasting interval ≥3 hours and body mass index <27.

glucose and insulin response found in women with gestational diabetes was present in patients with only minor glucose abnormality, i.e., only aberrant results of the glucose challenge test (the abnormal group in this study). As there were insufficient cases of diabetes in our original sample to have the statistical power necessary for an adequate comparison, data were gathered from the current literature for this evaluation.

Coustan et al.[3] found a significantly lower plasma glucose screening value when the 50 gm challenge was

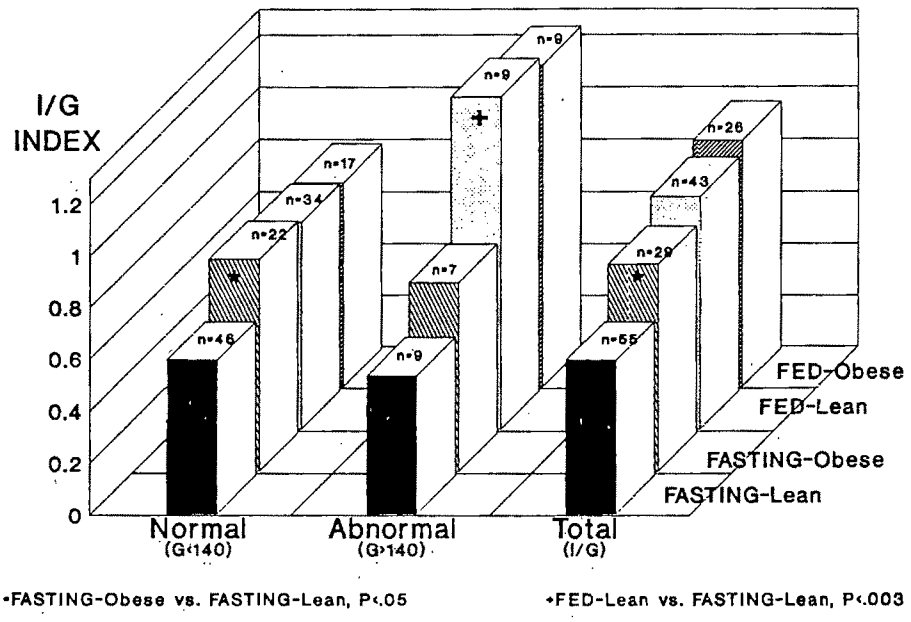


Fig. 3. Effect of fasting interval and obesity on insulin/glucose index. *FED-Obese,* Fasting interval <3 hours and body mass index ≥27. *FED-Lean,* Fasting interval <3 hours and body mass index <27. *FASTING-Obese,* Fasting interval ≥3 hours and body mass index ≥27. FASTING-Lean, Fasting interval ≥3 hours and body mass index <27.

administered 1 hour after a standard 600 kcal meal was eaten, but this was true only in subjects known to have gestational diabetes. They hypothesized that this lower value occurred in the "fed" state as compared to the "fasting" state because eatng had "dampened" the normal insulin resistance. We found the insulin resistance, as reflected by the insulin/glucose ration, to be increased in the "fed" mothers in our abnormal group, but there was no difference in the glucose response of "fed" versus "fasting" gravid women. Thus patients with only an abnormal glucose challenge test behaved similarly to the "presumed normal" patients in the study of Coustan et al., i.e., with no difference in glucose response, but they had increased insulin values probably because of the protein present in their last meal. A dampened insulin resistance was not seen; rather the "fed" subjects with gestational diabetes most likely received stimulation to produce more insulin. This lowered the glucose level significantly, but not to the level of the normal patients, as reflected by higher glucose values (154.8 ± 24.1 vs 115.8 ± 23.4 gm/dl) in that study.[5]

In a well-done study by Nuttall et al.[6] untreated, nonpregnant individuals with diabetes were given mixed protein and glucose meals. With increasing protein supplement, they found greater insulin response without any increase in glucose level. Clearly, screening soon after a mixed meal, including protein, as may have occurred in the "fed" group of this study, could explain the increased insulin response obtained. This effect of protein would necessarily be prolonged in pregnancy, as a result of the increased gastric emptying time, and this physiologic phenomenon might explain the 3-hour stratification found in this report.

Cheney et al.[7] studied obese and nonobese women with gestational diabetes after a standard 400 kcal mixed meal. They found significantly increased glucose and insulin levels for obese patients with gestational diabetes but decreased insulin levels in lean women with gestational diabetes when compared with normal subjects. They concluded that the obese patients with gestational diabetes had increased insulin resistance, whereas the lean patients with gestational diabetes had insulin deficiency. Catalano et al.[9] showed that this resistance was present even after delivery when obese subjects with gestational diabetes were tested with the hyperinsulinemic-euglycemic clamp. In our study the "fed" patients are similar to the post-mixed-meal patients of Cheney et al. In the abnormal group, glucose, insulin, and insulin/glucose index (i.e., insulin resistance) were increased as compared with values in normal gravid women. This was true for both obese and lean patients in this group. Thus the lean patients with minor glucose abnormalities in our study did not show any deficiency in insulin response.

A number of investigators[10-14] have shown that insulin sensitivity declines when an individual becomes obese. Ryan et al.[15] demonstrated, by means of euglycemic glucose clamp techniques, that nondiabetic pregnant patients have increased insulin resistance over non-

pregnant ones, and gestational-onset diabetic women have even greater resistance. The findings in this study of increased insulin and insulin/glucose index in obese patients in the "fasting" group are consistent with these findings. The relatively greater increase in insulin response of nonobese patients, when "fed," seemed to overshadow the effect of obesity, even in the group with glucose values ≥140 mg/dl, where both obese and lean patients have increased insulin output. Thus the "fed" state increased the insulin response to a greater extent than obesity altered insulin resistance in these mothers with minimal glucose abnormalities.

In summary, when compared with patients who undergo a standard oral 50 gm glucose challenge test after a fasting interval of >3 hours, pregnant women who have eaten more recently ("fed" women) have a greater insulin response if the glucose screening level is ≥140 mg/dl, whereas obese subjects have evidence of increased insulin resistance only when "fasting," regardless of glucose level.

REFERENCES

1. American College of Obstetricians and Gynecologists. Management of diabetes in pregnancy. Washington: ACOG, 1986; ACOG Tech Bull no 92.
2. O'Sullivan JB, Mahan CM, Dandrow RV. Screening criteria for high-risk gestational diabetic patients. AM J OBSTET GYNECOL 1973;116:895-900.
3. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
4. Summary and recommendations of workshop-conference on gestational diabetes. Diabetes Care 1980;3:499-501.
5. Coustan DR, Widness JA, Carpenter MW, et al. Should the fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state? AM J OBSTET GYNECOL 1986;154:1031-5.
6. Nuttall FQ, Mooradian AD, Gannon MC, Billington C, Krezowdki P. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. Diabetes Care 1984;7:465-70.
7. Cheney C, Shragg P, Hollingsworth D. Demonstration of heterogeneity in gestational diabetes by a 400-kcal breakfast meal tolerance test. Obstet Gynecol 1985;65:17-23.
8. Haffner SM, Stern MP, Hazuda HP, Pugh JA, Patterson JK. Hyperinsulinemia in a population at high risk for non-insulin-dependent diabetes mellitus. N Engl J Med 1986; 315:220-4.
9. Catalano PM, Bernstein IM, Wolfe RR, et al. Subclinical abnormalities of glucose metabolism in subjects with previous gestational diabetes. AM J OBSTET GYNECOL 1986; 1155:1255-62.
10. DeFronzo RA, Sherwin RS, Hendler R, Felig P. Insulin binding to monocytes and insulin action in human obesity, starvation, and refeeding. J Clin Invest 1978;62:204-13.
11. Bonadonna R, Groop L, Kraemer N, DeFronzo RA. Obesity and insulin resistance in man: a dose response study. Metabolism [In press].
12. Golay A, Felber JP, Jequier E, DeFronzo RA, Ferrannini E. Metabolic basis of obesity and non-insulin dependent diabetes mellitus. Diabetes Metab Rev 1988;4:727-47.
13. Kolterman OG, Insel J, Saekow M, Olefsky JM. Mechanism of insulin resistance in human obesity—evidence for receptor and post-receptor defects. J Clin Invest 1980; 65:1273-84.
14. Bogardas C, Lillioja S, Mott D, Reaven GR, Kashiwagi A, Foley J. Relationship between obesity and maximal insulin-stimulated glucose uptake in vivo and in vitro in Pima Indians. J Clin Invest 1984;73:800-5.
15. Ryan E, Brower E, O'Sullivan M, Skyler J. Insulin resistance in pregnancy. Studies with the euglycemic clamp technique. Diabetes 1985;34:380.

Relationships between glucose levels and insulin secretion during a glucose challenge test

Michael D. Berkus, MD,[a] Michael P. Stern, MD,[b] Braxton D. Mitchell, PhD,[b] Aida Abashawl, BS,[b] and Oded Langer, MD[a]

San Antonio, Texas

The relationship between glucose and insulin levels was examined in a prospective study of 153 pregnant patients without diabetes who underwent a standard 50 gm glucose challenge test. One hundred eighteen women had normal screening results (glucose level <140 mg/dl) and 35 had abnormal screening values but a normal oral glucose tolerance test. Abnormal responders had greater insulin levels (149 vs 82 pmol/L, $p < 0.0001$), and a higher insulin/glucose index (0.96 vs 0.72, $p < 0.007$). Patients with glucose levels <100 mg/dl had significantly lower insulin/glucose indices. Overall, obese patients had significantly greater glucose and insulin measurements than did nonobese women, but there was no difference within normal and abnormal groups. Glucose levels accounted for 52% of the insulin output and 29% of the insulin/glucose index variance. Neither age, parity, nor obesity contributed significantly to insulin levels in the multiple regression model. Therefore the accepted threshold for glucose screening reflects abnormal insulin output and this aberration may be indicative of the primary defect in gestational diabetes. (Am J Obstet Gynecol 1990;163:1818-22.)

Key words: Diabetes screening, minimal glucose abnormality, insulin output

The concept of screening for gestational diabetes with the glucose challenge test is well established.[1-4] In addition, there appears to be a positive association between the glucose challenge test and glucose profile in pregnancy, with a significantly higher profile in the abnormal screening group. However, minimal information exists as to the basic endocrinologic significance of an abnormal glucose challenge test.

It has become axiomatic that obesity is associated with diabetes as a result of insulin resistance.[5-8] It is this resistance that may result in both type II diabetes and gestational diabetes mellitus. Tissue sensitivity to insulin has been found to decline by approximately 30% to 40% when an individual exceeds 35% to 40% of ideal body weight.[7] This insulin resistance primarily affects muscle and involves both oxidative and nonoxidative pathways of glucose disposal. However, the unanswered question remains: What is the effect of obesity on insulin secretion in minor degrees of glucose abnormality in pregnancy, reflected by an abnormal glucose challenge test in the presence of a normal oral glucose tolerance test?

From the Department of Obstetrics and Gynecology[a] and the Department of Medicine,[b] The University of Texas Health Science Center at San Antonio.

Supported in part by National Institutes of Health grant HL-24799.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, March 21-24, 1990, St. Louis, Missouri.
Received for publication May 18, 1990; revised July 18, 1990; accepted August 6, 1990.

Reprint requests: Michael D. Berkus, MD, Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836.

6/6/24763

The purpose of this study was to examine the effect of glucose level on insulin output during a glucose challenge test, to ascertain the additional influence of obesity on this output, and to determine whether alterations in glucose and insulin values previously reported in gestational diabetes[9, 10] are present in cases with only minor degrees of glucose abnormality.

Material and methods

Nonhypertensive pregnant women were recruited from the obstetrics clinic of The University of Texas Health Science Center at San Antonio and from other San Antonio area clinics. The subjects were between 24 and 28 weeks' gestation or late registrants after 28 weeks and were not receiving insulin at the time of the study. The subjects included women with presumed normal pregnancies about to undergo screening for gestational diabetes. The study was approved by the institutional review board of the university and all subjects gave informed consent.

Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. For the purpose of analysis, patients were considered to be obese if their body mass index was ≥27. A patient's glucose screening was considered to be normal if the values were <140 mg/dl and abnormal if the values were greater; the patient was considered to have gestational diabetes mellitus when at least two values on the oral glucose tolerance test were abnormal. All testing was completed during morning clinics.

Blood samples were drawn 1 hour after a 50 gm load of a glucose equivalent (Koladex or Orangedex, Cus-

Table I. Patient characteristics by glucose screening level

	Group 1: Normal (<140 mg/dl) (n = 118)	*Group 2: Abnormal (≥140 mg/dl) (n = 35)*	*p Value*
Age	23.8 ± 5.7	25.6 ± 6.1	NS
Parity			
Primigravid (%)	12	6	NS
Multiparous (%)	88	94	NS
Hispanic (%)	71	80	NS
Gestational age at screening (wk)	30 ± 5	30 ± 5	NS
1 hr glucose (mg/dl)	110 ± 16	156 ± 12	—
Obesity (%)	33	49	NS
Insulin (pmol/L)	81.5 ± 48	149.3 ± 94	0.0001
Insulin/glucose index	0.72 ± 0.4	0.96 ± 0.6	0.007

tom Laboratories, Baltimore) was administered. Plasma glucose was determined by the glucose oxidase method and was measured with a Paramax Chemistry Analyzer (American Dade Chemical Systems, Irvine, Calif.). Serum insulin concentrations were measured with a commercial solid-phase radioimmunoassay procedure (Diagnostic Products, Los Angeles). The coefficient of variation between duplicate aliquots measured at the same time was 8.1% for the 1-hour concentrations of insulin.[11] The insulin/glucose index was calculated to adjust for differing glucose levels.

Data analysis included bivariate and multivariate statistical techniques with parametric and nonparametric tests. Categoric data were analyzed with Fisher's exact and χ^2 tests. Continuous data were analyzed by the Student t test. One-way analysis of variance was used to compare different levels of glycemia. The association between variables was determined by calculating Pearson's correlation coefficients.

A stepwise multiple regression was performed to determine the extent to which the variables contributed to insulin secretion. Both tests of significance and the magnitude of the relationships, expressed as the variance of the dependent variable that could be accounted for by the independent variable, were used to report regression results. Variables contributing 3% of the total variance were considered worthwhile. In all cases, the F values were significant at the 0.01 level.

Results

A total of 171 patients participated in this prospective study. The group consisted of pregnant patients undergoing a routine 50 gm glucose screening for gestational diabetes, as outlined by the American College of Obstetricians and Gynecologists.[12] Patients were enrolled on an availability basis. Missing data were as follows: Four patients did not obtain the glucose screening and four additional subjects did not have insulin levels measured. A total of 163 complete data sets were available for analysis. There were 118 women with normal glucose screens (<140 mg/dl) and 45 with glucose values ≥140 mg/dl. Of the 45 subjects with abnormal screens, 10 had gestational diabetes mellitus. Those women with gestational diabetes mellitus were excluded from the analysis.

Table II. Analysis of variance of the level of glycemia (by screening results) and insulin response

Glucose (mg/dl)	*n*	*Insulin (pmol/L)*	*Insulin/glucose index*
<100	32	46.9 ± 33*	0.52 ± 0.3†
100-119	47	82.4 ± 43‡	0.76 ± 0.4
120-139	39	108.8 ± 48§	0.85 ± 0.4
140-159	24	145.1 ± 68	0.97 ± 0.5
>160	11	158.5 ± 137	0.92 ± 0.8

*Insulin: (Glucose <100) versus (Glucose ≥100), $p < 0.01$.

†Insulin/glucose index: (Glucose <100) versus (Glucose ≥100), $p < 0.02$.

‡Insulin: (Glucose 100 to 119) versus (Glucose ≥120), $p < 0.04$.

§Insulin: (Glucose 120 to 139) versus (Glucose ≥140), $p < 0.02$.

There were no significant differences in demographic characteristics between the normal and abnormal glucose groups (Table I). The abnormal responders had a greater insulin level, as would be expected; in addition, they had significantly greater insulin/glucose index values.

Examination of levels of glycemia of all subjects, by means of analysis of variance for 20 mg/dl groupings, yielded significant differences for insulin ($F = 14.22$) and insulin/glucose index ($F = 4.60$) (Table II). Subsequent posthoc t tests between mean insulin values showed significantly lower values between each of the glucose groupings <140 mg/dl and higher glucose levels, but no differences for the groups ≥140 mg/dl. The t tests between mean insulin/glucose index values generated significantly lower values for only the group of women with glucose values <100 mg/dl. Thus this subgroup of low glucose reactors had relative hypoinsulinemia.

Overall, obese gravid women had significantly greater glucose and insulin serum levels than did non-

Table III. Association between insulin secretion and maternal size

	Nonobese (n = 97)	*Obese (n = 56)*	*p Value*
Insulin (pmol/L)	87.1 ± 60	114.2 ± 76	0.02
Insulin/glucose index	0.72 ± 0.4	0.87 ± 0.5	0.053
Glucose	117 ± 24	127 ± 25	0.01

obese subjects, but only a marginally greater insulin/glucose index (Table III). Although there were significant differences in insulin levels between groups for both obese and nonobese women, obesity had no differential effect on the insulin/glucose index within the groups, and only a marginally significant increase in glucose and insulin values for women with glucose levels <140 mg/dl (Table IV).

There was a relatively strong association between glucose and insulin level as determined by Pearson correlation ($r = 0.54$, $p < 0.0001$), and a significant but less strong association between glucose and insulin/glucose index ($r = 0.31$, $p < 0.0001$), glucose and body mass index ($r = 0.21$, $p < 0.01$), and body mass index and insulin level ($r = 0.17$, $p < 0.04$). There was no association between body mass index and insulin/glucose index.

Stepwise multiple regression was performed to determine the relative contribution of glucose, body mass index, parity, and age to insulin values. Only the level of glycemia had a significant F value, and accounted for 52% of the total variance in insulin level and 29% of the insulin/glucose index variance. None of the other variables, including body mass index, contributed significantly to the insulin values in the multiple regression model.

Comment

The major finding of this study was that patients with abnormal glucose screening during a 50 gm glucose challenge test had increased insulin output, even after adjustment for glucose values. This was true for both the normal and abnormal screening groups. There was a significantly lower insulin value among subjects with screening glucose values <100 mg/dl. The insulin levels within the group with values <140 mg/dl were otherwise indistinguishable, as were the responses of subjects with glucose levels >140 mg/dl. Furthermore, multivariant analysis failed to show any contribution of age, parity, or maternal size to the insulin values of either group.

Increased insulin resistance in patients with type II diabetes has been shown conclusively by use of insulin-clamp techniques.[13] The same methods have been used to demonstrate increasing resistance in pregnancy: nondiabetic pregnant patients have increased resistance over nonpregnant women, and women with gestational onset diabetes have even greater impairment.[14] Researchers also have found increased insulin resistance that persisted post partum in obese women with gestational diabetes mellitus even when standard measures of glucose tolerance were normal.[10] Whether these differential effects are present during a pregnancy with only minor degrees of glucose abnormality has not been addressed.

The results of this study support the concept that there is heightened insulin output in patients with abnormal glucose challenge test results over pregnant patients with glucose values <140 mg/dl, even if the oral glucose tolerance test is normal. The increased insulin/glucose index also was consistent with previous reports of increased insulin resistance. Bivariate analysis yielded only a marginally significant contribution of obesity to glucose and insulin measurements. However, this relationship was not independent of glucose level because multivariate analysis failed to show any additional contribution of maternal size to insulin secretion or resistance, as evidenced by the insulin/glucose index in patients with minimal glucose abnormality. In addition, there was relative hypoinsulinemia among the patients with glucose screening values <100 mg/dl. In this group, there was significantly less insulin measured, even when adjusted for the glucose values. This may represent compensatory mechanisms to facilitate glucose transfer to the fetus, even in mothers with low levels of serum glucose. There are recent reports that high-risk patients with hypoglycemic-hypoinsulinemia are at increased risk for small-for-gestational-age infants.[15]

Although there is evidence that maternal age is a risk factor for gestational diabetes mellitus,[12] no such association was found in this investigation for patients with lesser glucose aberration. Current research has shown a slight increase in the risk of noninsulin-dependent diabetes mellitus or impaired glucose tolerance with increasing parity many years after childbearing, independent of obesity.[16] Neither of these factors contributed significantly to the insulin output in this study during pregnancy. Thus there was no evidence that these variables had an impact on insulin levels during the glucose challenge test in women with only minor degrees of glucose abnormality, with only glucose level remaining as the main determinant.

The threshold of 140 mg/dl for glucose abnormality in the glucose challenge test is well established.[3, 4, 12] The underlying endocrinopathy of that recommendation is not so apparent. Our study attempted to uncover the

Table IV. Association between maternal size, glucose screening level, and insulin response

	Nonobese	*Obese*	*p Value*
Normal (<140 mg/dl)	$n = 79$	$n = 39$	
Insulin (pmol/L)	75.5 ± 44	93.7 ± 54	0.056
Insulin/glucose index	0.68 ± 0.4	0.8 ± 0.4	NS
Glucose (mg/dl)	108 ± 16	114 ± 16	0.06
Abnormal (≥140 mg/dl)	$n = 18$	$n = 17$	
Insulin (pmol/L)	138 ± 89	161 ± 98	NS
Insulin/glucose index	0.89 ± 0.6	1.02 ± 0.6	NS
Glucose (mg/dl)	155 ± 12	158 ± 12	NS

basis for this threshold and provide a physiologic rationale for the choice of such a value. The differential insulin production discovered in the abnormal screening group, present in even minimal glucose divergence, may offer an explanation.

Recently it has been proposed by several investigators that even "relative hyperglycemia" in nondiabetic subjects is associated with an increased adverse outcome, primarily macrosomia. Tallarigo et al.[17] found that even limited degrees of maternal hyperglycemia were associated with a significant increase in the incidence of macrosomia, congenital abnormalities, toxemia, and cesarean section. Langer et al.[18] found that gravid subjects with one abnormal value on an oral glucose tolerance test had daily glucose profiles comparable to those of women with gestational diabetes mellitus, and this profile was significantly higher than that of normal nondiabetic mothers. Moreover, the incidence of macrosomia has been found to be significantly greater in patients with one abnormal glucose value when compared with treated subjects with gestational diabetes mellitus (24% vs 6%,[18] 18% vs 6.6%[19]). Leikin et al.[20] also determined that patients with minor abnormalities of carbohydrate metabolism (glucose levels ≥135 mg/dl on a glucose challenge test) were at increased risk for delivery of a macrosomic infant.

Therefore it is evident that even minor glucose abnormalities are associated with fetal macrosomia, a classic complication related to glycemic level, as proposed by the Pedersen theory.[21] Presumably, patients with these abnormalities have elevated insulin levels and probably increased insulin resistance. However, it has not been shown that these subjects have a basic insulinopathy. Our data are consistent with the existence of an insulin defect in even minor degrees of glucose deviation.

Consequently, this study offers evidence that glucose abnormality is a continuum associated with abnormal insulin levels that becomes increasingly evident with greater degrees of glucose aberration. Moreover, it appears that the current threshold for glucose screening does reflect significant differences in insulin output that may underlie the basic endocrinopathy of gestational diabetes mellitus. Further research is needed to substantiate these observations.

REFERENCES

1. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol 1982; 144:768-73.
2. O'Sullivan JB, Mahan CM, Dandrow RV. Screening criteria for high-risk gestational diabetic patients. Am J Obstet Gynecol 1973;116:895-900.
3. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
4. Summary and Recommendations of Workshop-Conference on Gestational Diabetes. Diabetes Care 1980; 3:499-501.
5. DeFronzo RA, Sherwin RS, Hendler R, Felig P. Insulin binding to monocytes and insulin action in human obesity, starvation, and refeeding. J Clin Invest 1978;62:204-13.
6. Bonadonna R, Groop L, Kraemer N, DeFronzo RA. Obesity and insulin resistance in man: a dose response study. Metabolism 1990;5:452-9.
7. Golay A, Felber JP, Jequier E, DeFronzo RA, Ferrannini E. Metabolic basis of obesity and non-insulin dependent diabetes mellitus. Diabetes Metab Rev 1988;4:727-47.
8. Kolterman OG, Insel J, Saekow M, Olefsky JM. Mechanism of insulin resistance in human obesity—evidence for receptor and post-receptor defects. J Clin Invest 1980; 65:1273-84.
9. Cheney C, Shragg P, Hollingsworth D. Demonstration of heterogeneity in gestational diabetes by a 400-kcal breakfast meal tolerance test. Obstet Gynecol 1985;65:17-23.
10. Catalano PM, Bernstein IM, Wolfe RR, et al. Subclinical abnormalities of glucose metabolism in subjects with previous gestational diabetes. Am J Obstet Gynecol 1986; 155:1255-62.
11. Haffner SM, Stern MP, Hazuda PH, Pugh JA, Patterson JK. Hyperinsulinemia in a population at high risk for non-insulin-dependent diabetes mellitus. N Engl J Med 1986; 315:220-4.
12. American College of Obstetricians and Gynecologists. Management of diabetes in pregnancy. Washington: American College of Obstetricians and Gynecologists, 1986; ACOG Tech Bull 92.
13. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;232:E214-23.
14. Ryan E, Brower E, O'Sullivan M, Skyler J. Insulin resistance in pregnancy: studies with the euglycemic clamp technique. Diabetes 1985;34:380-4.
15. Langer O, Damus K, Maimon M, Divon M, Levy J, Bauman W. A link between relative hypoglycemia-hypoinsulinemia during oral glucose tolerance tests and intrauterine growth retardation. Am J Obstet Gynecol 1986;155:711-6.

16. Kritz-Silverstein D, Barrett-Connor E, Wlingard DL. The effect of parity on the later development of non-insulin-dependent diabetes mellitus or impaired glucose tolerance. N Engl J Med 1989;321:1214-9.
17. Tallarigo L, Giampietro O, Penno G, Miccoli R, Gregoria G, Navalesi R. Relation of glucose tolerance to complications of pregnancy in nondiabetic women. N Engl J Med 1986;315:989-92.
18. Langer O, Anyaegbunam A, Brustman L, Divon M. Management of women with one abnormal oral glucose tolerance test value reduces adverse outcome in pregnancy. Am J Obstet Gynecol 1989;161:593-9.
19. Lindsay MK, Graves W, Klein L. The relationship of one abnormal glucose tolerance test value and pregnancy complications. Obstet Gynecol 1989;73:103-6.
20. Leikin EL, Jenkins JH, Pomerantz GA, Klein L. Abnormal glucose screening tests in pregnancy: a risk factor for fetal macrosomia. Obstet Gynecol 1987;69:570-3.
21. Pedersen J, ed. The pregnant diabetic and her newborn. Problems and management. 2nd ed. Baltimore: Williams & Wilkins, 1977:211-20.

Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: Results of a controlled, randomized, prospective study

Siri L. Kjos, MD, Donna Shoupe, MD, Sylvie Douyan, MD, Richard L. Friedman, MD, Gerald S. Bernstein, MD, Jorge H. Mestman, MD, and Daniel R. Mishell, Jr., MD
Los Angeles, California

Women with recent gestational diabetes mellitus were randomly assigned to one of two low-dose oral contraceptives to evaluate the effect of low-dose oral contraceptives on carbohydrate and lipid metabolism. A cohort of similar women requesting a non-oral-contraceptive method served as controls. The two oral contraceptives studied were ethinyl estradiol (0.035 mg)–norethindrone (0.40 mg) and ethinyl estradiol (0.030 to 0.040 mg)–levonorgestrel (0.050 to 0.125 mg). A 75 gm, 2-hour oral glucose tolerance test and a fasting lipid profile (total cholesterol, triglyceride, high- and low-density lipoprotein cholesterols) were performed at entry, after 3 months, and after 6 to 13 months of treatment. The prevalence of diabetes at 6 to 13 months (27/156 patients) was not significantly different between groups (non-oral-contraceptive group, 17%; ethinyl estradiol–norethindrone, 15%; ethinyl estradiol–levonorgestrel, 20%). When examined by prior gestational diabetes mellitus class, diabetes mellitus was present in 7% of prior class A_1 and 29% of women with prior class A_2 disease ($p < 0.001$). Mean cholesterol and low-density lipoprotein cholesterol levels were significantly improved in all three groups at 3 months and at 6 to 13 months, whereas triglycerides remained unchanged. There were no differences in cholesterol, low-density lipoprotein cholesterol, or triglycerides levels between the groups. After 6 to 13 months, there was a significant increase in high-density lipoprotein cholesterol in the ethinyl estradiol–norethindrone group compared with the ethinyl estradiol–levonorgestrel and non-oral-contraceptive groups. (Am J Obstet Gynecol 1990;163: 1822-7.)

Key words: Gestational diabetes mellitus, low-dose oral contraceptives, lipids, glucose tolerance

Determination of the metabolic effect of oral contraceptives in women with prior gestational diabetes is complicated by inherent deterioration in carbohydrate tolerance. Within 15 years of the diagnosis of gestational diabetes mellitus, diabetes mellitus will develop in more than half of these women, with those who required insulin during pregnancy at greatest risk.[1-3] Earlier reports of high-dose oral contraceptive use in women with prior gestational diabetes mellitus show marked deterioration in glucose tolerance.[4,5] The dose and potency of the progestin component of oral contraceptives appear to decrease glucose tolerance, increase insulin resistance, and adversely affect cholesterol, whereas estrogen appears to counterbalance these changes.[6-9] By decreasing both the estrogen and

From the Department of Obstetrics and Gynecology, University of Southern California Medical School.
Supported in part by a grant from Mead Johnson Laboratories.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Siri L. Kjos, MD, Room 5K-40, LAC–USC Women's Hospital, 1240 North Mission Road, Los Angeles, CA 90033.
6/6/24769

progestin dose, newer low-dose oral contraceptives have little or no effect on carbohydrate and lipid metabolism in healthy women.[10-16] Two recent 6-month studies address low-dose oral contraceptive use in women with prior gestational diabetes mellitus. One study shows no change in glucose tolerance, insulin, or lipid levels,[17,18] whereas the other demonstrates an increase in glucose and insulin levels.[19]

This study evaluates the effect of short-term usage (6 to 13 months) of two randomly assigned low-dose oral contraceptives on carbohydrate and lipid metabolism in postpartum women with recent gestational diabetes mellitus. The metabolic status of these women is compared with that of a similar cohort of women who also had recent gestational diabetes mellitus, but who chose form of birth control other than OCs. This study design allows the determination of whether postpartum deterioration of carbohydrate or lipid metabolism is inherently related to the disease process alone or whether there is an additional deleterious effect from oral contraceptive use.

Material and methods

Subjects. A total of 230 patients were studied. All had recent gestational diabetes mellitus diagnosed by two or more abnormal values during a 100 gm 3-hour oral glucose tolerance test during pregnancy.[20] Of these women, 95% were of Latin American origin or descent. Patients were subclassified according to their degree of fasting hyperglycemia during pregnancy. Patients with class A_1 disease (N = 124) maintained fasting serum glucose levels below 105 mg/dl. Patients with class A_2 disease (N = 106) had at least one fasting serum glucose level ≥105 mg/dl.[21] Postpartum patients underwent entry glucose tolerance testing (mean time from delivery to entry test, 49 days). Those patients diagnosed with diabetes mellitus were excluded from study entry. Patients desiring oral contraceptives were randomized to receive either a monophasic preparation of 0.40 mg norethindrone and 0.035 mg ethinyl estradiol (Ovcon 35, Mead Johnson, Evansville, Ind.) or a triphasic preparation of 0.050 to 0.125 mg levonorgestrel and 0.030 to 0.040 mg ethinyl estradiol (Triphasil, Wyeth, Philadelphia). These two preparations were chosen because they contain the lowest amount of the two progestins most commonly used in the United States. Patients who chose alternative methods were enrolled as non-oral-contraceptive controls without being randomized. Non-oral-contraceptive methods included barrier methods, the intrauterine contraceptive device, and tubal sterilization. All patients were counseled to continue the American Diabetes Association diet. The study protocol was approved by the Research Committee of the Los Angeles County–University of Southern California Medical Center.

Study design. Metabolic studies, consisting of a 2-hour oral glucose tolerance test and a fasting lipid profile (cholesterol, triglyceride, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol), were performed at entry, 3 months, and 6 to 13 months. Pill compliance, weight, and blood pressure were recorded at each visit. The diagnosis of diabetes mellitus at 3 months constituted a study end point that necessitated the discontinuation of oral contraceptives. These patients were not retested at 6 to 13 months.

Metabolic studies. After 3 days of a diet that provided at least 150 gm/day of carbohydrate, patients fasted overnight and then ingested 75 gm of glucose over 5 minutes. Blood specimens were obtained by venipuncture at −5, +30, +60, +90, and +120 minutes. Specimens were transported on ice for immediate separation of serum and cells. Serum was stored at −20° C for subsequent glucose measurement (model II glucose analyzer, Beckman Instruments, Fullerton, Calif.). Results were interpreted according to the National Diabetes Data Group definitions of diabetes mellitus and impaired glucose tolerance.[20] Glucose tolerance between study groups was also evaluated by integrating the total area under the glucose tolerance curve (gm · min/dl) over the 120-minute oral glucose tolerance test. Total cholesterol and triglyceride levels were measured by procedures that use enzymatic hydrolysis and oxidation (Olympus AU5061, Olympus Corp., New York). High-density lipoprotein cholesterol was determined by procedures using cholesterol oxidase, after sodium phosphotungsate precipitation of low-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol (Beckman CX4). Low-density lipoprotein cholesterol was calculated as (Low-density lipoprotein cholesterol = Cholesterol − [high-density lipoprotein cholesterol + triglyceride/5]).[22]

Statistical analysis. Continuous variables (area under the curve, lipids, weight, etc.) within a study group or gestational diabetes mellitus class over time were analyzed by paired one-sample analysis; differences between groups were examined with two-sample analysis and analysis of variance. Differences between study groups or gestational diabetes mellitus classes with respect to glucose tolerance test status were tested for significance by χ^2, with adjustments for multiple comparisons when appropriate.

Results

Table I shows the demographic data comparing the study groups at entry. The non-oral-contraceptive patients are slightly older, heavier, and of higher parity. The mean body mass index (calculated as kg/m^2) is elevated in all three groups; a standard definition of overweight was used as body mass index ≥ 27 kg/m^2.[23] The percentage of the patients with prior class

Table I. Entry demographic data according to study birth control methods

	Study birth control method		
	Non-oral-contraceptive group (N = 67)	*Ethinyl estradiol–norethindrone (N = 80)*	*Ethinyl estradiol–levonorgestrel (N = 83)*
Age (yr)	31 ± 4	28 ± 5	29 ± 5
BMI (kg/m²)	31 ± 6*	29 ± 5	28 ± 5
Parity	3 ± 2	2 ± 1	2 ± 1
Class A_2	55%	38%	45%

Data recorded are mean ± SD. *BMI*, Body mass index—Weight/Height)². The non-oral-contraceptive methods included barrier methods, intrauterine contraceptive devices, and sterilization. The monophasic preparation consisted of 0.40 mg norethindrone and 0.035 mg ethinyl estradiol, and the triphasic preparation consisted of 0.050-0.125 mg levonorgestrel and 0.030 to 0.040 mg ethinyl estradiol.

*$p < 0.05$. For all other methods, p = ns.

A_2 gestational diabetes mellitus is not significantly different between the groups. The interval in days from the delivery data to the 6 to 13 months evaluation for each of the study groups was not significantly different (non-oral-contraceptive, 343 ± 120 days; ethinyl estradiol–norethindrone, 317 ± 102 days; ethinyl estradiol–levonorgestrel, 346 ± 149 days). This is important because the risk of developing diabetes after a pregnancy complicted by gestational diabetes mellitus increases with time after delivery.[1, 2]

No patients had hypertension (≥140/90 mm Hg) that necessitated discontinuation of oral contraceptives. Oral contraceptives were discontinued in four women after 3 months of use because of markedly elevated triglyceride levels (≥500 mg/dl). All four had elevated triglyceride levels post partum (≥300 mg/dl) that did not respond to diet therapy. One hundred fifty-six patients completed the testing at 6 to 13 months. Seventy women did not complete the study at 6 to 13 months because of self-discontinuation of oral contraceptives or loss to follow-up. These patients did not differ significantly in mean age (29 ± 5 years), parity (3 ± 1), body mass index (29 ± 5 kg/m²), entry area under the curve values (16.2 ± 4.0 gm · min/dl), or percent composition of prior class A_2 patients (42%).

Glucose tolerance. Table II shows the mean total area under the curve at entry, 3 months, and 6 to 13 months according to study groups. There was no significant difference in mean area under the curve between the three groups at each testing interval (entry, 3 months, and 6 to 13 months). When the data were analyzed within a group as a function of time, there was an increase in area under the curve during the study period. Paired one-sample analysis showed a significant increase in area under the curve for both oral contraceptive groups at 3 months and 6 to 13 months when compared with their entry area under the curve ($p < 0.04$). There was a similar, nonsignificant trend ($p = 0.06$) in the non-oral-contraceptive group. This analysis of area under the curve omitted the results of 6 to 13-month testing of patients with the highest 3-month area under the curve; women diagnosed with diabettes mellitus, as these 14 women (five in non-oral-contraceptive group, three in the ethinyl estradiol–norethindrone group, and six in the ethinyl estradiol–levonorgestrel group), were not retested.

The clinically more useful method of interpreting these findings was to use the glucose tolerance test status at 6 to 13 months, including the data of the 14 women diagnosed as having diabetes mellitus at 3 months (Table III). The prevalence of impaired glucose tolerance and diabetes mellitus was not significantly different among the three groups, irrespective of oral contraceptive use. In the non-oral-contraceptive patients, impaired glucose tolerance and diabetes mellitus each occurred in nine (17%) patients. Impaired glucose tolerance and diabetes mellitus were respectively present in five (11%) and seven (15%) patients in the ethinyl estradiol–norethindrone group and in eight (14%) and 11 (20%) patients in the ethinyl estradiol–levonorgestrel group. The overall prevalence of diabetes was 17% by 6 to 13 months.

Analysis of the 6- to 13-month oral glucose tolerance test status by gestational diabetes mellitus classification rather than by birth control method showed a significant relationship (Table IV). Both impaired glucosetolerance and diabetes mellitus were significantly more prevalent at 6 to 13 months in women with prior class A_2 gestational diabetes mellitus ($p = 0.03$ and $p < 0.001$, respectively). Among women in class A_2, 14 of 73 (19%) had impaired glucose tolerance and 21 (29%) had diabetes mellitus, whereas in patients with class A_1 disease, eight of 83 (10%) had impaired glucose tolerance and six (7%) had diabetes mellitus. A difference in carbohydrate metabolism between patients with class A_1 and A_2 disease was also evident at the entry test. In patients with prior class A_2 disease at entry, impaired glucose tolerance was found in 23 of 106 (21%) compared with eight of 124 (6.4%) of patients with prior class A_1 disease ($p < 0.001$). The entry area under the

Table II. Numbers of patients tested at each interval and mean values (± SD) at testing intervals (at entry, after 3 months and after 6 to 13 months) according to study birth control method

	Entry	*Testing interval*	
		3 mo	*6 to 13 mo*
No. of patients tested			
Non-oral-contraceptive method	67	29	53
Ethinyl estradiol–norethindrone	80	65	47
Ethinyl estradiol–levonorgestrel	83	75	56
Area under curve			
Non-oral-contraceptive method	17.2 ± 3.6	19.5 ± 5.9	18.2 ± 4.7*
Ethinyl estradiol–norethindrone	16.2 ± 3.9	17.7 ± 3.9†	17.8 ± 4.2†
Ethinyl estradiol–levonorgestrel	16.9 ± 3.6	18.1 ± 4.2†	18.5 ± 4.7†
Total cholesterol			
Non-oral-contraceptive method	229 ± 49	202 ± 40‡	199 ± 34‡
Ethinyl estradiol–norethindrone	208 ± 37	192 ± 39‡	192 ± 33‡
Ethinyl estradiol–levonorgestrel	217 ± 37	199 ± 34‡	190 ± 33‡
Low-density lipoprotein cholesterol			
Non-oral-contraceptive method	133 ± 56	121 ± 42§	120 ± 32‖
Ethinyl estradiol–norethindrone	123 ± 38	98 ± 45§	105 ± 28‖
Ethinyl estradiol–levonorgestrel	129 ± 38	111 ± 37§	111 ± 28‖
High-density lipoprotein cholesterol			
Non-oral-contraceptive method	47 ± 13	44 ± 9	46 ± 10
Ethinyl estradiol–norethindrone	45 ± 11	47 ± 13	52 ± 7‖¶
Ethinyl estradiol–levonorgestrel	47 ± 12	47 ± 12	47 ± 9
Triglyceride			
Non-oral-contraceptive method	201 ± 144	179 ± 120	168 ± 109
Ethinyl estradiol–norethindrone	185 ± 89	199 ± 105	177 ± 70
Ethinyl estradiol–levonorgestrel	190 ± 108	198 ± 131	161 ± 72

See Table I for definitions of study groups. The p values denote the statistical significance of changes in measured parameters, compared with entry values, within a study group. For all unsymboled values, p = ns.

*$p = 0.06$.

†$p < 0.04$.

‡$p < 0.001$.

§$p \leq 0.05$.

‖$p < 0.01$.

¶A significant intergroup difference in high-density lipoprotein cholesterol exists: $p \leq 0.01$ versus non-oral-contraceptive and ethinyl estradiol–levonorgestrel high-density lipoprotein cholesterol at 6 to 13 months. Other differences between study groups within a testing interval are not significant.

Table III. Prevalence of normal, impaired, and diabetic glucose tolerance after 6 to 13 months of study birth control method in women with recent gestational diabetes mellitus

	6-13 mo oral glucose tolerance test status		
	Normal	*Impaired*	*Diabetic*
Non-oral-contraceptive method (n = 53)	35 (66%)	9 (17%)	9 (17%)
Ethinyl estradiol–norethindrone (n = 47)	35 (74%)	5 (11%)	7 (15%)
Ethinyl estradiol–levonorgestrel (n = 56)	37 (66%)	8 (14%)	11 (20%)
TOTAL	107 (69%)	22 (14%)	27 (17%)

Study groups are defined in Table I. Numbers in parentheses denote number of women who completed the study, including 14 women diagnosed with diabetes mellitus after 3 months of study. There are no significant intergroup differences in prevalence of glucose tolerance test status (p = 0.48).

curve of women with prior class A_2 gestational diabetes mellitus was correspondingly higher (18.4 ± 3.3 compared with 15.4 ± 3.0 gm · min/dl) ($p < 0.001$).

Cholesterol. There was a significant decrease in mean total cholesterol levels in all study groups when the 3-month and the 6- to 13-month levels were compared with the mean entry cholesterol level ($p < 0.001$, Table III). There was no significant difference between the study groups at each testing interval. The overall mean cholesterol at 6 to 13 months was 194 ± 34 mg/dl.

Low-density lipoprotein cholesterol. There was also a significant decrease in mean low density lipoprotein cholesterol levels at 3 months and at 6 to 13 months

Table IV. Prevalence of normal, impaired, and diabetic glucose tolerance after 6 to 13 months of study according to prior gestational diabetes classification

	6-13 mo oral glucose tolerance test status		
	Normal	*Impaired*	*Diabetic*
Class A_1 (n = 83)	69 (83%)	8 (10%)	6 (7%)
Class A_2 (n = 73)	38 (52%)	14* (19%)	21† (29%)
TOTAL	107 (69%)	22 (14%)	27 (17%)

Class A_1 and A_2 denote women with antepartum fasting serum glucose levels <105 and ≥105 mg/dl respectively. Numbers in parentheses denote the number of women in each group.

*p = 0.03 versus class A_1 impaired glucose tolerance.

†$p < 0.001$ versus class A_1 diabetes mellitus.

compared with entry levels in all three study groups ($p \leq 0.05$ and $p \leq 0.01$, respectively). The overall mean low density lipoprotein cholesterol level at 6 to 13 months was 121 ± 31 mg/dl.

High-density lipoprotein cholesterol. The mean high-density lipoprotein cholesterol was unchanged in both the non-oral-contraceptive and ethinyl estradiol–levonorgestrel groups throughout the study. In contrast, the 6- to 13-month mean high-density lipoprotein cholesterol level was significantly higher in the ethinyl estradiol–norethindrone group when compared with the mean entry high-density lipoprotein cholesterol level ($p < 0.01$). The mean high-density lipoprotein cholesterol level in this group was also significantly higher compared with the 6- to 13-month levels in the non-oral-contraceptive and ethinyl estradiol–levonorgestrel groups ($p \leq 0.01$).

Triglycerides. There was no significant difference in mean triglyceride levels either within each group over time or between groups at each testing interval.

Abnormal glucose tolerance test status (impaired glucose tolerance or diabetes mellitus) was not associated with a higher mean cholesterol, low density lipoprotein cholesterol, triglyceride, or with a decreased mean high-density lipoprotein cholesterol level at any testing interval. The study population was overweight (mean body mass index, 29 ± 5). There was no significant weight change from entry during the study period in any of the groups. Although there was a trend toward increasing mean body mass index in patients with impaired glucose tolerance and diabetes mellitus at all testing intervals, these associations were not significant.

Comment

In this study population low-dose oral contraceptives did not influence the development of diabetes mellitus in women with recent gestational diabetes mellitus. These patients were overweight and primarily of Latin American descent or origin. Both these factors are associated with glucose intolerance.[24] Gestational diabetes mellitus class has a major influence on the development of subsequent diabetes. In this study only 7% of patients with class A_1 diabetes were seen to have diabetes mellitus compared with 29% of patients with class A_2 diabetes. The deterioration in glucose tolerance was closely related to the inherent disease process of gestational diabetes mellitus, rather than use of oral contraceptives.

Elevation of lipids during pregnancy is well documented.[25, 26] A delay in the return to normal levels longer than 6 weeks post partum has been reported.[27] The improvement of lipid profiles by 3 months in all of our study groups suggests a similar gradual return of lipid metabolism to a nonpregnant state in women with prior gestational diabetes mellitus. The mean cholesterol and low density lipoprotein cholesterol levels at 6 to 13 months in these study patients are comparable with those in the Department of Health and Human Services 1978-1980 survey for a similar age cohort of women (25 to 34 years old) in which the mean cholesterol and low density lipoprotein cholesterol levels were 192 ± 34 and 121 ± 33 mg/dl, respectively.[28, 29] The increase after 6 to 13 months of ethinyl estradiol–norethindrone use in high-density lipoprotein cholesterol is likely related to its estrogen-dominant oral contraceptive formulation.

Short-term use of low-dose oral contraceptives in women with prior gestational diabetes mellitus does not appear to have any adverse effect on carbohydrate or lipid metabolism. Both oral contraceptive groups and the non-oral-contraceptive group show a significant and similar deterioration in glucose tolerance with an overall prevalence of 14% impaired glucose tolerance and 17% diabetes mellitus after 6 to 13 months. Low-dose oral contraceptives can be safely prescribed in women with prior gestational diabetes mellitus. Serum lipids and glucose tolerance should be monitored closely regardless of contraceptive choice. The ability to offer these patients an effective, acceptable, and reversible birth control method such as the low-dose OC increases the ability to prevent or plan pregnancies in

accordance with optimal maternal glycemic status and desires. More studies are needed to determine the long-term effect of low-dose oral contraceptive use as well as the natural history of disease progression in patients with gestational diabetes.

We thank the staff and practitioners of Women's Hospital Family Planning Clinic for their dedication and help in caring for these patients and Rebecca Burt, RN, for her assistance as the Diabetes Family Planning Nurse.

REFERENCES

1. O'Sullivan JB. Long term follow up of gestational diabetics. In: Camerini-Davalos RA, Cole HS, eds. Early diabetes in early life. New York: Academic Press, 1975:503-10.
2. Mestman JH. Follow-up studies in women with gestational diabetes mellitus. The experience at Los Angeles County/University of Southern California Medical Center. In: Weiss PAM, Coustan DR, eds. Gestational diabetes. New York: Springer-Verlag, 1987:191-8.
3. Metzger BE, Bybee DE, Freinkel N, et al. Gestational diabetes mellitus. Correlations between the phenotypic and genotypic characteristics of the mother and abnormal glucose tolerance during the first year postpartum. Diabetes 1985;34(suppl 2):111-5.
4. Szabo AJ, Cole HS, Grimaldi RD. Glucose tolerance in gestational diabetic women during and after treatment with a combination type oral contraceptive. N Engl J Med 1970;282:646-50.
5. Beck P, Wells SA. Comparison of the mechanisms underlying carbohydrate intolerance in subclinical diabetic women during pregnancy and during postpartum oral contraceptive steroid treatment. J Clin Endocrinol Metab 1969;29:807.
6. Gaspard UJ. Metabolic effects of oral contraceptives. Am J Obstet Gynecol 1987;157:1029-41.
7. Perlman JA, Russell-Briefel R, Ezzati T, et al. Oral glucose tolerance and the potency of contraceptive progestins. J Chronic Dis 1985;38:857-64.
8. Kalkhoff RK. Effects of oral contraceptive agents on carbohydrate metabolism. J Steroid Biochem 1975;6:949-56.
9. Spellacy WN, Buhi WC, Birk SA. The effect of estrogens on carbohydrate metabolism: glucose, insulin, and growth hormone studies on ingesting Premarin, mestranol, and ethinyl estradiol for six months. Am J Obstet Gynecol 1972;114:378-92.
10. Spellacy WN, Buhi WC, Birk SA, et al. Carbohydrate metabolism studies in women using Brevicon, a low estrogen oral contraceptive for one year. J Reprod Med 1981;26:295-8.
11. Spellacy WN, Bird SA, Buggie J, et al. Prospective carbohydrate metabolism studies in women using a low estrogen oral contraceptive for one year. J Reprod Med 1981;24:415-27.
12. Luyckx AS, Gaspard UJ, Romus MA, et al. Carbohydrate metabolism in women who used oral contraceptives containing levonorgestrel or desogestrel: a 6-month prospective study. Fertil Steril 1986;45:635-42.
13. Knopp RH, Walden CE, Wahl PW, et al. Effects of oral contraceptives on lipoprotein triglyceride and cholesterol: relationship to estrogen and progestin potency. Am J Obstet Gynecol 1982;142:725-31.
14. Gaspard UJ, Burlt J, Gillain D, et al. Serum lipid and lipoprotein changes induced by new oral contraceptives containing ethinylestradiol plus levonorgestrel or desogestrel. Contraception 1985;31:395-408.
15. van del Vange N, Kloosterboer HJ, Haspels AA. Effect of seven low-dose combined oral contraceptive preparations on carbohydrate metabolism. Am J Obstet Gynecol 1987;156:918-22.
16. Vermeulen A, Thiery M. Metabolic effects of the triphasic oral contraceptive Trigynon. Contraception 1982;26:505-13.
17. Skouby SO, Kuhl C, Molsted-Pedersen L, et al. Triphasic oral contraception: metabolic effects in normal women and those with previous gestational diabetes. Am J Obstet Gynecol 1985;153:495-500.
18. Skouby SO, Anderson O, Saurbrey, et al. Oral contraception and insulin sensitivity: in vivo assessment in normal women and women with previous gestational diabetes. J Clin Endocrinol Metab 1987;64:519-23.
19. Kung AWC, Ma JTC, Wong VCW, et al. Glucose and lipid metabolism with triphasic oral contraceptives in women with history of gestational diabetes. Contraception 1987;35:257-69.
20. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
21. American College of Obstetrics and Gynecology. Management of diabetes mellitus in pregnancy. ACOG Tech Bull May 1986 no 92.
22. Friedwald WT, levy RI. Frederickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
23. National Institutes of Health consensus development panel on the health implications of obesity. Ann Intern Med 1985;103(6 pt 2):1073-7.
24. Haffner SM, Stern MP, Hazuda HP, et al. Role of obesity and fat distribution in non-insulin dependent diabetes mellitus in Mexican Americans and non-Hispanic whites. Diabetes Care 1986;9:153-60.
25. Warth MR, Arky RA, Knopp RH. Lipoprotein metabolism in pregnancy. III. Altered lipid composition in intermediate, very low, low and high density lipoprotein fractions. J Clin Endocrinol Metab 1975;41:649-55.
26. Knopp RH, Warth M. Lipoprotein changes in pregnancy. J Clin Invest 1973;52:48a.
27. Darmady JD, Postle AD. Lipid metabolism in pregnancy. Br J Obstet Gynaecol 1982;89:211-5.
28. National Center for Health Statistics. Total serum cholesterol levels of adults 20 to 74 years of age: United States, 1976-1980. Washington: US Government Printing Office, May 1986, DHHS publication no (PHS)86-1686. (Vital and health statistics, series 2, no 236).
29. Report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults. Arch Intern Med 1988;148:36-69.

Response of placental vasculature to high glucose levels in the isolated human placental cotyledon

Jonathan B. Roth, MD, James A. Thorp, MD, Sue M. Palmer, MD, Peter C. Brath, BS, Scott W. Walsh, PhD, and Sharon S. Crandell, MD

Houston, Texas

We report the effect of high glucose infusion on vascular resistance in isolated human placental cotyledons perfused with Krebs-Ringer–bicarbonate solution containing 80 mg/dl (4.4 mmol/L), 160 mg/dl (8.8 mmol/L), or 320 mg/dl (17.6 mmol/L) D-glucose (n = 6). Placental vascular resistance remained constant during 25-minute perfusion periods with 80 mg/dl followed by 160 mg/dl glucose solution. Subsequent perfusion with 320 mg/dl glucose produced a significant increase in placental vascular resistance. Placentas were also studied in which the placental cotyledon was sequentially perfused for 25-minute periods with solutions containing glucose at 80 mg/dl followed immediately by 320 mg/dl (n = 5). Placental vascular resistance remained constant throughout perfusion with 80 mg/dl glucose solution but increased significantly after beginning perfusion with 320 mg/dl glucose. We conclude that the increase in placental vascular resistance appears to be a function of the high glucose level rather than the duration of glucose perfusion. (AM J OBSTET GYNECOL 1990;163:1828-30.)

Key words: Placenta, glucose, vascular resistance, diabetes

Maternal hyperglycemia results in fetal hyperglycemia, fetal hyperinsulinemia, and an increased flux of glucose into the fetal circulation. These metabolic alterations appear to have a deleterious effect on fetal well-being. Phillips et al.[1] showed that fetal hyperglycemia produced by glucose infusion into the ovine fetus resulted in increased oxygen consumption, hypoxemia, acidemia, and fetal death in fetuses that became severely acidemic. In studying the effects of maternal hyperglycemia on the fetus, we previously showed that the hyperglycemic fetus became hypoxemic and a mixed metabolic respiratory acidemia developed.[2] In addition, umbilical blood flow was significantly decreased at maternal glucose concentrations that were two to three times the normal range for pregnant ewes. The cause of this observed decrease in umbilical blood flow during maternal-fetal hyperglycemia was unclear.

Fetal hypoxemia is a potent stimulus for the release of vasoactive substances that may contribute to reduced placental blood flow. Rankin and Phernetton[3, 4] reported that norepinephrine and prostaglandin E_2 caused vasoconstriction in the placental vascular bed and thereby decreased umbilical blood flow.[4] Angiotensin II increases placental vascular resistance.[5] It is not clear by what mechanism the decrease in umbilical blood flow is initiated or sustained during maternal-fetal hyperglycemia, or what if any role fetal hypoxemia plays in the redistribution of fetal cardiac output and whether this redistribution is mediated by the release of vasoactive agents. Despite extensive in vivo work in glucose metabolism in chronically catheterized ovine fetuses, few investigators have examined directly the effect of glucose on placental vascular resistance. The following in vitro study was undertaken to examine the effects of high glucose levels on vascular resistance in the human placenta.

Material and methods

The method used involved simultaneous in vitro perfusion of the placental vasculature and the intervillous space in the isolated human placental cotyledon as previously described by Schneider et al.[6] and Thorp et al.[7] The placentas were obtained immediately after vaginal or cesarean section delivery and placed into a bath of physiologic saline solution at room temperature. An intact lobe on the fetal side of the placenta suitable for dual perfusion was selected and the arterial and venous chorionic vessels supplying that lobe were catheterized. The placenta was then secured onto a chamber and two butterfly needles were pierced through the decidual

From the Perinatal Physiology Research Unit, Departments of Pediatrics, Obstetrics, Gynecology, and Reproduction Sciences, and Physiology and Cell Biology, University of Texas Medical School at Houston.
Supported in part by Grant No. HD 20973 from the National Institute of Child Health and Human Development.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Jonathan B. Roth, MD, Presbyterian Hospital, P.O. Box 33549, 200 Hawthorne Lane, Charlotte, NC 28233.
6/6/24748

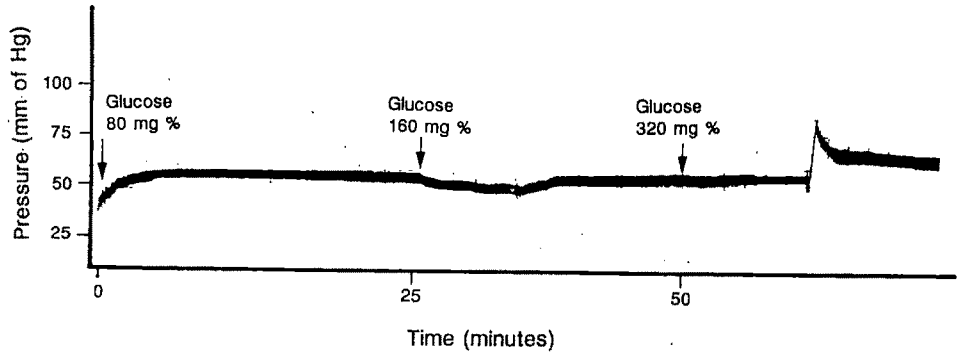


Fig. 1. Representative tracing of change in pressure of chorionic plate artery during perfusion of isolated human placental cotyledon with sequentially increasing concentrations of glucose (80 mg/dl (4.4 mmol/L), 160 mg/dl (8.8 mmol/L), and 320 mg/dl (17.6 mmol/L).

plate of the maternal side of the placenta. The fetal flow was set at 2 to 5 ml/min and the maternal flow at 7 to 12 ml/min. The maternal and fetal sides of the placenta were perfused with a Krebs-Ringer–phosphate–bicarbonate solution gased with 95% air and 5% carbon dioxide at 37° C. Pressure changes in the chorionic plate placental arteries and veins were monitored with a physiologic recorder (model 7754B, Hewlett Packard, Waltham, Mass.). Changes in resistance were calculated by dividing the difference between arterial and venous pressures by the measured flow rates.

We studied six placentas (group A). The placental cotyledons were sequentially perfused for 25-minute periods with solutions that contained glucose of 80 mg/dl (4.4 mmol/L), 160 mg/dl (8.8 mmol/L), and 320 mg/dl (17.6 mmol/L). In addition, five placentas were studied in which the placental cotyledons were sequentially perfused for 25-minute periods with solutions that contained glucose at 80 mg/dl (4.4 mmol/L) followed by 320 mg/dl (17.6 mmol/L) (group B). Solutions of mannitol of 80 mg/dl (4.4 mmol/L), 160 mg/dl (8.8 mmol/L), and 320 mg/dl (17.6 mmol/L) were used to perfuse two additional placentas. Data were analyzed as appropriate by the paired Student *t* test or analysis of variance with random block due to variability between placentas. Tissue viability was determined in six placentas by measuring lactate production after 15 minutes, 60 minutes, and 120 minutes of perfusion.

Results

There was no significant change in lactate production throughout the experiment, indicating that the tissue remained viable during experimental procedures. Mean lactate production at 15 minutes was 0.79, at 60 minutes 0.89, and at 120 minutes 0.97 mmol/min/gm. These values are within the range reported by others.[8] Placental vascular resistance remained constant throughout a 25-minute perfusion with 80 mg/dl glucose solution and throughout a 25-minute perfusion with 160 mg/dl glucose solution. Within 12 ± 3 minutes of beginning the perfusion with 320 mg/dl glucose, a significant increase of 110% ($p < 0.05$) in placental vascular resistance was observed (Fig. 1, Table I).

Table I. Placental vascular resistance in response to perfusion with different concentrations of glucose studied in the isolated human placental cotyledon

	Resistance (mm Hg · min/ml)	
Glucose (mg/dl)	*Group A mean ± SE (n = 6)*	*Group B mean ± SE (n = 5)*
80	14.17 ± 4.26	16.82 ± 3.56
160	14.26 ± 4.22	—
320	30.00 ± 11.4	31.49 ± 5.25

In an additional five placentas studied, vascular resistance remained constant throughout a 25-minute perfusion with 80 mg/dl glucose solution. Within 11 ± 3 minutes of beginning the perfusion with 320 mg/dl glucose, a significant increase of 87% in placental vascular resistance was observed ($p < 0.01$) (Fig. 2, Table I). In the two placentas perfused with mannitol, vascular resistance remained constant throughout sequential perfusions with mannitol solutions of 80 mg/dl, 160 mg/dl, and 320 mg/dl, which shows that the vasoconstriction observed with 320 mg/dl glucose was not simply an osmotic effect.

Comment

Placental vascular resistance increased in response to high glucose levels. The absence of a response in the placentas perfused with mannitol indicates that this re-

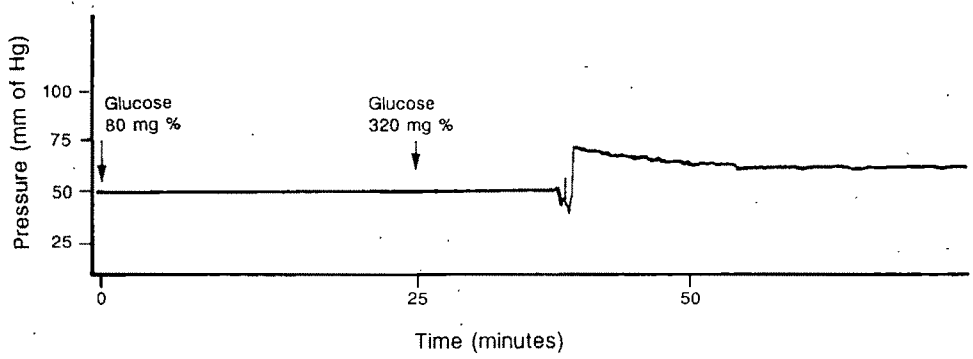


Fig. 2. Same as Fig. 1, except the 320 mg/dl glucose perfusion immediately followed the 80 mg/dl glucose perfusion.

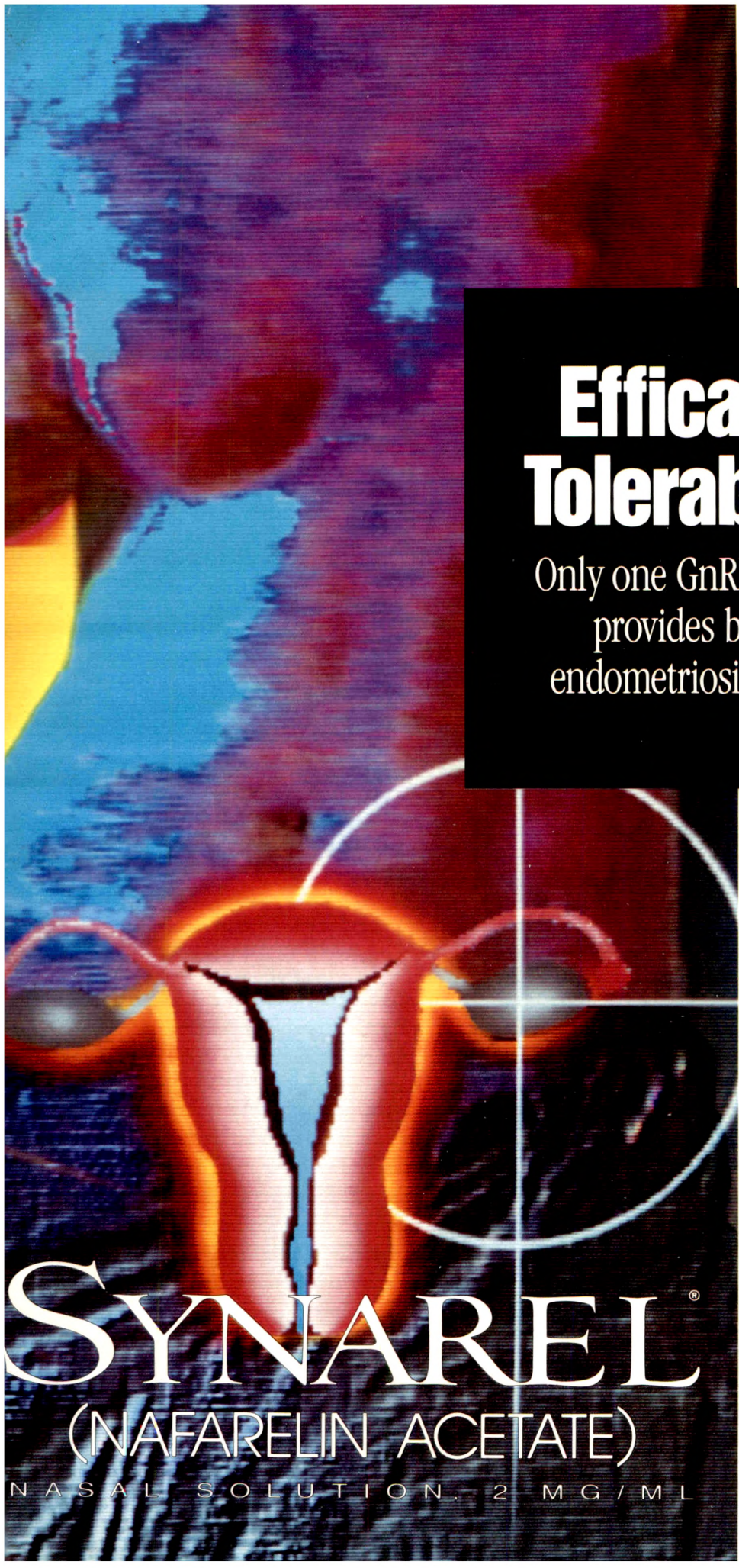
sponse is not an osmotic effect. In addition, the increase in placental vascular resistance appears to be a function of the high glucose level and not a result of a time-related effect of the glucose perfusion. Because there are differences in the anatomic relationship of the perfusing vessels in the sheep versus the human placenta, one cannot be certain that high glucose levels would produce similar vasoconstriction in the sheep placenta. The data do, however, suggest that the decrease in umbilical blood flow observed by Crandell et al.[2] in the intact pregnant sheep may have resulted from changes in resistance in the placental vascular bed as a consequence of maternal hyperglycemia.

The mechanism for the increase in placental vascular resistance after perfusion with high glucose levels is not known. In vivo, fetal hyperglycemia causes hypoxemia. Fetal hypoxemia is a potent stimulus for the release of vasoactive substances that may contribute to increased placental vascular resistance. Fetal hypoxemia evokes the release of vasoconstrictors such as vasopressin, renin, epinephrine, norepinephrine, and prostaglandin E_2 and $F_{2\alpha}$ into the circulation.[3,4,5,9,10] However, this cannot explain vasoconstriction in response to high glucose levels in vitro. High glucose levels caused a decrease in prostacyclin production by cultured trophoblast cells obtained from early pregnancy,[11] and there is a significant decrease in prostacyclin production with no significant change in thromboxane production by placentas obtained from women with diabetes as compared with nondiabetic women.[12] Therefore, vasoconstriction in response to high glucose levels may be related to decreased prostacyclin production leading to an imbalance between thromboxane and prostacyclin levels that favors the vasoconstrictive effects of thromboxane.

We speculate that the increase in placental vascular resistance observed in vitro may explain in vivo the decrease in umbilical blood flow during maternal-fetal hyperglycemia observed in our laboratory in the sheep and by Trudinger et al.[13] in the human uncontrolled diabetic patient.

REFERENCES

1. Phillips AF, Porte PJ, Stabiusky S, Rosenkrantz TS, Raye JR. Effects of chronic fetal hyperglycemia upon oxygen consumption in the ovine uterus and conceptus. J Clin Invest 1984;74:279-86.
2. Crandell SS, Fisher DJ, Morriss FH. Effects of ovine maternal hyperglycemia on fetal regional blood flows and metabolism. Am J Physiol 1985;249:E454-60.
3. Rankin JGH, Phernetton TM. Effect of norepinephrine on the ovine umbilical circulation. Proc Soc Exp Biol Med 1976;152:312-7.
4. Rankin JHG, Phernetton TM. Circulatory responses of the near-term sheep fetus to prostaglandin E_2. Am J Physiol 1976;D231:760-5.
5. Iwamoto HS, Rudolph AM, Keo! LC, Heymann MA. Hemodynamic response of the sheep fetus to vasopressin infusion. Circ Res 1976;44:430-6.
6. Schneider H, Panigel M, Dancis J. Transfer across the perfused human placenta of antipyrine, sodium, and leucine. Am J Obstet Gynecol 1972;114:822-8.
7. Thorp JA, Walsh SW, Brath PC. Comparison of the vasoactive effects of leukotrienes with thromboxane mimic in the perfused human placenta. Am J Obstet Gynecol 1988;159:1376-80.
8. Bloxam DL, Bullen BE. Condition and performance of the perfused human placental cotyledon. Am J Obstet Gynecol 1986;155:382-8.
9. Robiland JE, Weitzman RE, Burmenster L, Smith FG. Development aspects of renal response to hypoxemia in the lamb fetus. Circ Res 1981;48:128-38.
10. Ferris TF, Stein JH, Kauffman J. Uterine blood flow and uterine renin secretion. J Clin Invest 1972;51:2827-33.
11. Rakoczi I, Tihanyi K, Gero G, Czeh I, Rozsa I, Gati I. Release of prostacyclin (PGI_2) from trophoblast in tissue culture: the effect of glucose concentration. Acta Physiol Hung 1988;71:545-9.
12. Walsh SW, Parisi VM. The role of prostanoids and thromboxane in the regulation of placental blood flow. In: Rosenfeld C, ed. The uterine circulation. Ithaca, New York: Perinatology Press, 1989:273-98.
13. Trudinger BJ, Giles WB, Cook CM, Bombardieri J, Collius L. Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol 1985;92:23-30.



Efficacy & Tolerability:

Only one GnRH* agonist provides both in endometriosis therapy

SYNAREL®

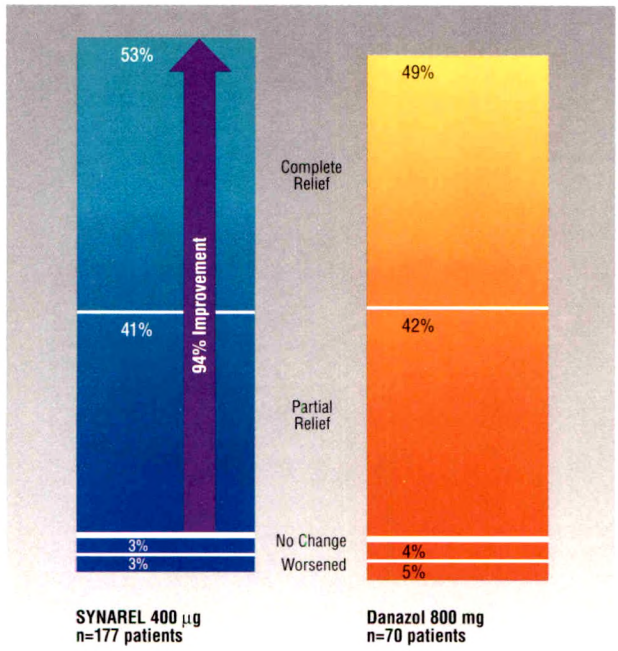
(NAFARELIN ACETATE)

NASAL SOLUTION, 2 MG/ML

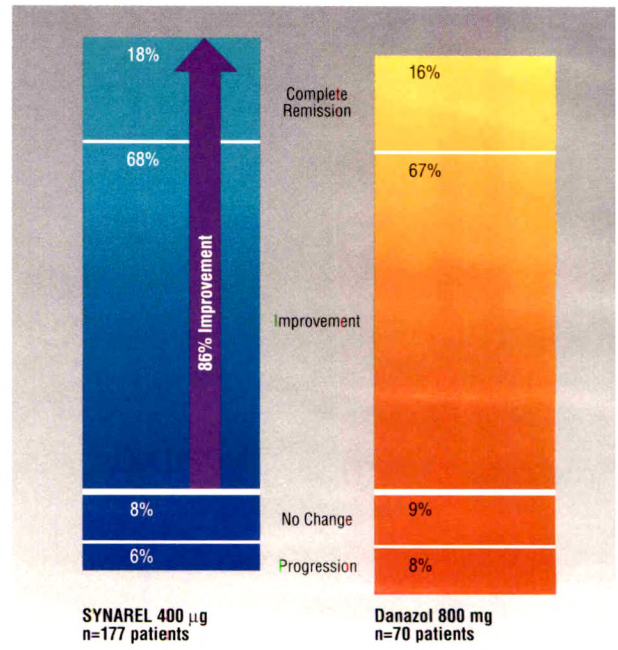
*Gonadotropin-releasing hormone analog
Please see brief summary of prescribing information on last page.

The Only GnRH* Agonist That Satisfies The Needs Of Endometriosis Therapy

Unsurpassed Resolution Of Pain And Endometriotic Lesions



Change in symptom severity scores from admission to end of study



Change in laparoscopy scores from admission to end of therapy

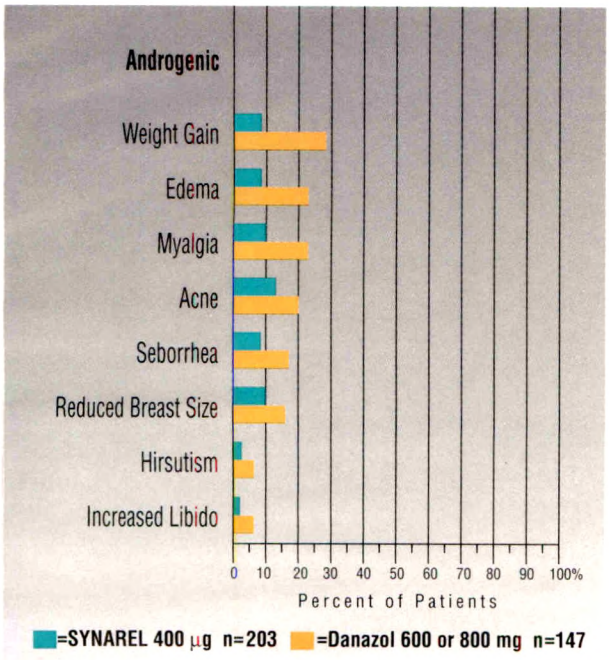
94% of Synarel patients reported improvement of symptoms

- Proven to relieve dysmenorrhea, dyspareunia, and pelvic pain
- Efficacy sustained in 83% of patients at six months posttreatment

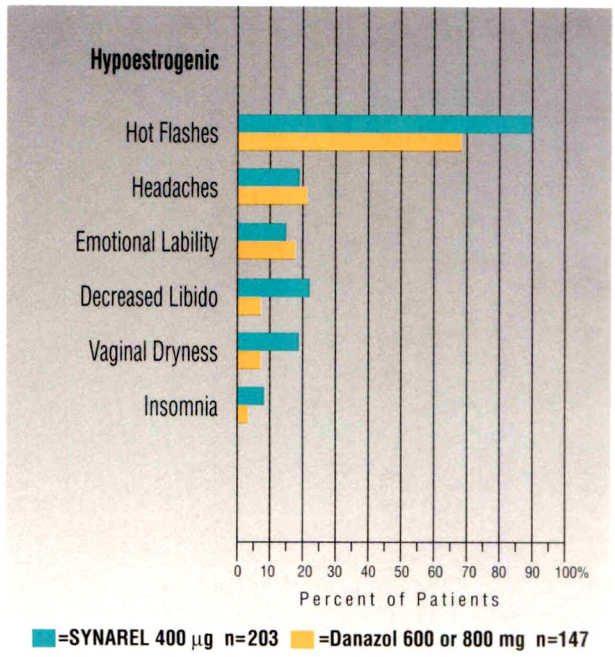
86% of Synarel patients showed improvement of endometriotic lesions

- Improvement in all stages of endometriosis confirmed by post-laparoscopy scores†
- Retreatment cannot be recommended since safety data for retreatment are not currently available

Well-Tolerated Management Of Endometriosis, An Estrogen-Dependent Disease



Adverse androgenic effects during six months of treatment



Adverse hypoestrogenic effects during six months of treatment

- Fewer masculinizing side effects than danazol
- Some androgenic effects of danazol, such as deepening of the voice, may be irreversible[1]
- Unlike danazol, NO increase in LDL/HDL ratio after six months of treatment[2]

- A predictable hypoestrogenic profile: Estrogen levels return to normal after cessation of therapy
- Pregnancy should be ruled out before therapy with SYNAREL is initiated. Patients should be advised to use a nonhormonal method of contraception when taking SYNAREL.



SYNAREL®

(NAFARELIN ACETATE)

NASAL SOLUTION, 2 MG/ML

Endometriosis therapy breakthrough

Please see brief summary of prescribing information on last page.

Gonadotropin-releasing hormone analog

Based on American Fertility Society laparoscopy score stages: (absent, score 0); I (minimal, 1–5); II (mild, 6–15); III moderate, 16–40); IV (severe, 40+).

The Only GnRH* Agonist Indicated For The Management Of Endometriosis

- Unsurpassed resolution of pain and endometriotic lesions
- Well-tolerated management of this estrogen-dependent disease
- Fewer masculinizing side effects than danazol (The most frequent side effects are those associated with hypoestrogenism.)
- The only GnRH agonist administered intranasally—assures reliable dosing control

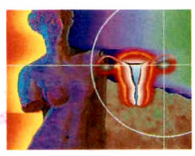
For more information, please call:
1-800-727-0400



1. Dickey RP: *Managing Danazol Patients.* Durant, OK, Creative Infomatics, Inc, 1985.
2. Henzl MR, Corson SL, Moghissi K, et al: Administration of nasal nafarelin as compared with oral danazol for endometriosis. *N Engl J Med* 1988;318:485–489.

*Gonadotropin-releasing hormone analog.

© 1990 Syntex Laboratories, Inc.
829-H2-206-90



SYNAREL®
(NAFARELIN ACETATE)
NASAL SOLUTION, 2 MG/ML

Endometriosis therapy breakthrough

BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloric glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.

INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and old treated for 6 months.

CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undia nosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL ma cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patie becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women wh are breast feeding.

WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pre nancy must be excluded.

When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Cont ception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal cc traception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregna during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.

PRECAUTIONS Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient shou notify her physician if regular menstruation persists. Patients missing successive doses may experience breakthrouc bleeding.

2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or a allergic to any of its ingredients.

3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should b used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consu her physician.

4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritatic occurred in about 10% of patients.

5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of th loss may not be reversible. During one six-month treatment period, this should not be important. In patients with maj risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNARE may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatme with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content.

6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topic nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrea the possibility of reducing drug absorption.

7. Retreatment cannot be recommended since safety data beyond 6 months are not available.

Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin aceta is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound plasma proteins at 4°C, therefore drug interactions would not be expected to occur.

Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. N mal function is usually restored 4–8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituita gonadotropic and gonadal functions during treatment and up to 4–8 weeks after discontinuation of SYNAREL may l misleading.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats a mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal do respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolong periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the inciden of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There w an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treat groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there w a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose femal No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potent

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment w discontinued after continuous administration for up to 6 months.

Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to ra throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on t relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/ fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 6 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administrati throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fe weight with the highest dose.

Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determin SYNAREL should not be used by nursing mothers.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse re tions were most frequently reported and thought to be drug-related.

Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20 myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depressi (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urtica asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be p sibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.

Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone ma measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, co pared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bo density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone ma measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total verteb mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if a decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than t recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause ac tional bone loss.

Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twi the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and leve returned to normal after treatment was stopped.

Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had to cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had pc treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNARE group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4. mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment valu were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of t patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) co pared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractio (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.

Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patien SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in seru calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.

OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analog

February 1990

© 1990 Syntex Laboratories, Inc.

Committed to the OB/GYN and women's healthcare

SYNTEX

SYNTEX LABORATORIES, INC
PALO ALTO, CA 94304

Placental thromboxane and prostacyclin production in an ovine diabetic model

Jan E. Dickinson, MD, Bruce A. Meyer, MD, Peter C. Brath, BS, Susie Chmielowiec, MS, Scott W. Walsh, PhD, Valerie M. Parisi, MD, MPH, and Sue M. Palmer, MD

Houston, Texas

We hypothesized that streptozocin-induced ovine diabetes would cause alterations in the placental production of thromboxane and prostacyclin. With a tissue incubation technique, we examined the placental production of thromboxane and prostacyclin in cotyledons from seven normal near-term ewes (127 ± 3 days' gestation) and six streptozocin-induced diabetic ewes (125 ± 3 days' gestation). Diabetic status was verified with serial fasting blood glucose assessments. Placental tissue was incubated in Dulbecco's modified Eagle's medium for 48 hours at 37° C with 95% oxygen and 5% carbon dioxide. Samples were collected at 0, 1, 2, 4, 8, 20, 32, and 48 hours. Radioimmunoassay of the stable metabolites thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ were used to determine thromboxane and prostacyclin production, respectively. Placental thromboxane production was reduced in diabetic animals when compared with control animals (5.63 ± 2.81 vs 7.32 ± 1.37 pg/mg per hour, respectively; $p < 0.05$). Prostacyclin production was also significantly reduced in the diabetic placentas compared with control placentas (11.44 ± 4.06 vs 16.29 ± 4.59 pg/mg per hour, respectively; $p < 0.05$). We conclude that the ovine placenta produces thromboxane and prostacyclin. The ovine thromboxane production rate is comparable to that of the human placenta but the prostacyclin production rate is approximately two to three times higher. The observed decrease in the placental production of thromboxane and prostacyclin may reflect an adverse effect of hyperglycemia directly on eicosanoid production or indirectly through decreased placental cellular proliferation. (Am J Obstet Gynecol 1990;163:1831-5.)

Key words: Prostacyclin, thromboxane, placenta, diabetes, ovine

During normal human pregnancy, the placenta produces approximately equal amounts of the vasoactive eicosanoids thromboxane and prostacyclin.[1, 2] Certain disease states are associated with alterations in the placental production of thromboxane and prostacyclin. Diabetes mellitus is a disease characterized by alterations in vascular physiology and eicosanoid production. Previous investigators showed reduced prostacyclin production from the endothelium of large blood vessels in nonpregnant diabetic subjects,[3, 4] and increased platelet thromboxane production.[5] These alterations in the production of eicosanoids appear to be glucose-related. Rakoczi et al.[6] showed a dose-dependent reduction of prostacyclin production in cultured trophoblast cells incubated with increasing glucose concentrations. Prostacyclin synthesis is reduced in the umbilical arteries of neonates of mothers with gestational diabetes mellitus and juvenile-onset diabetes.[7] Maternal gestational diabetes mellitus is associated with a decrease in the in vitro placental production rate of prostacyclin, but not that of thromboxane (Palmer SM, Young S, Walsh SW. Unpublished observations). The clinical implication of reduced placental prostacyclin production in gestational diabetes may be decreased placental blood flow, limited vasodilation, and increased platelet aggregation. These alterations in placental physiology may impact on the capacity of the fetus of the diabetic mother to respond to changes in uteroplacental perfusion.

Preeclampsia is a common complication of pregnancy in the diabetic patient, particularly in women with poor glucose control. In preeclampsia the placental production of the vasoconstrictor thromboxane is seven times that of the vasodilator prostacyclin, and this imbalance has been postulated to account for some of the pathophysiology of this disease.[1, 2] The frequency with which diabetes and preeclampsia are associated in pregnancy prompted our investigation of the placental production of eicosanoids in diabetes.

With the pregnant ewe, we investigated the role of the vasoactive eicosanoids in normal and diabetic pregnancies. We hypothesized that the ovine placenta produces thromboxane and prostacyclin, and that streptozocin-induced diabetes mellitus would alter the production rates of these eicosanoids.

From the Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School at Houston.
Supported by Grant Nos. HD-18699, HD-20973, and HD-00782.
Presented in part in the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Jan E. Dickinson, MD, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School at Houston, Suite 3.204, 6431 Fannin, Houston, TX 77030.
6/6/24747

Material and methods

Six pregnant ewes of known gestational age were rendered glucose intolerant by the intravenous administration of streptozocin (Upjohn Co., Kalamazoo, Mich.) on two separate occasions, 4 days apart, commencing at 85 to 95 days' gestation. Before each injection of streptozocin, a 3-hour intravenous glucose tolerance test with 25 gm of glucose administered as 50 ml of 50% dextrose in water was conducted. At the completion of the glucose tolerance test, streptozocin was administered to the ewe at a dose of 50 mg/kg maternal weight. Immediately before injection, the streptozocin was reconstituted in 50 ml of sterile 0.9% sodium chloride and then infused over 3 to 4 minutes via a maternal peripheral hind-limb vein. To ensure active secretion of insulin and maximal degranulation of the pancreatic β-cells, the streptozocin was injected while the animals were feeding. A third maternal intravenous glucose tolerance test was performed at 114 to 123 days' gestation, 5 days before surgery. Maternal venous plasma fasting blood glucose levels were assessed with a glucose analyzer (model 23A, Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio) before the first administration of streptozocin and again before surgery at 119 to 128 days' gestation.

At 112 to 121 days' gestation the ewes were transferred to the Animal Care Center at our institution and acclimated for a minimum of 7 days before surgery. A diet of pelleted alfalfa and hay was fed to the ewes. The animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, 1985) in the Animal Care Center of the University of Texas Medical School at Houston. The experimental protocol was approved by our institutional Animal Welfare Committee.

Placental cotyledons, approximately 3 cm in diameter, were obtained at the time of surgery from six glucose-intolerant (125 ± 3 days' gestation) and seven control sheep (127 ± 3 days' gestation). The cotyledon closest to the uterine incision site was selected and atraumatically excised, with double ligation of the vascular pedicle. After harvesting of the placental cotyledons in both glucose-intolerant and control ewes, the insertion of maternal and fetal vascular catheters was performed for the purpose of later experimentation with radioactive microspheres.

Placental tissues were immediately chilled and processed under sterile conditions at 4° C as previously described.[1,2] Placental tissues were separated, gently cut into thin-layer pieces, and washed repeatedly with Dulbecco's modified Eagle's medium (Gibco, Grand Island, N.Y.). Excess modified Eagle's medium was removed with sterile gauze. Multiple wells, each containing approximately 350 mg of placental tissue, were incubated in 7 ml of modified Eagle's medium for 48 hours at 37° C in a Dubnoff metabolic shaker. Tissues were oxygenated with 95% oxygen and 5% carbon dioxide. Samples of media (200 μl) were collected at 0, 1, 2, 4, 8, 20, 32, and 48 hours of incubation. Tissue viability after 0, 20, 32, and 48 hours of incubation was assessed by trypan blue dye exclusion staining as previously described.[2] Results were considered valid if tissue viability was >80%.

The production of thromboxane A_2 was estimated by determination of the concentration of its stable metabolite, thromboxane B_2, with a highly specific radioimmunoassay. The assay consisted of competitive binding of radioactive and natural thromboxane B_2 to a highly specific antibody. Dextran-coated charcoal was used to separate the bound from the free fraction. Cross-reactivity of the antibody with other eicosanoids was <0.1%. Within-assay variation was 5.8% and between-assay variation was 14.0% ($n = 6$). The least detectable concentration was 0.7 ± 0.2 pg, and 50% binding of the standard curve was 16.5 ± 1.3 pg ($n = 6$). Serial sample dilutions were parallel to the standard curve, and the addition of increasing amounts of thromboxane B_2 to modified Eagle's medium resulted in a linear dose response ($r^2 = 1.00$, $y = 1.08x + 0.00$, $n = 6$). A zero dose response resulted from modified Eagle's medium alone. The production of prostacyclin was determined by specific radioimmunoassay of its stable metabolite, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). The radioimmunoassay method and its validation were performed in the same manner to that described for thromboxane.[1] The within-assay variation was 10.9% and between-assay variation was 13.1% ($n = 3$). The least detectable concentration was 0.8 ± 0.2 pg, and 50% binding of the standard curve was 30.1 ± 2.4 pg ($n = 3$). As with thromboxane, the addition of increasing amounts of 6-keto-$PGF_{1\alpha}$ to modified Eagle's medium showed a linear dose response ($r^2 = 0.9993$, $y = 1.10x - 8.5$, $n = 3$). All data are mean ± SE.

Statistical analysis was performed by independent Student's t test (comparison of production rates), simple linear regression (calculation of production rates) and two-way analysis of variance with random block (comparison of the concentration regression lines) with a computer statistical software package (Abstat, Anderson-Bell, Parker, Colo.). Logarithmic transformation [log (x + 1)] was used when variances were not equal. All data are mean ± SE. A probability of $p < 0.05$ was chosen to represent statistical significance.

Results

After streptozocin administration, the fasting venous plasma glucose values of the six ewes rose significantly from a pretreatment level of 71.4 ± 17.0 mg/dl to

121.7 ± 43.0 mg/dl 4 weeks later, $p < 0.05$ (Fig. 1). Maternal venous plasma glucose values have been reported for the normal pregnant ewe and show a gradual decrease as gestation advances. Normoglycemia for the late gestation ewe is 63.8 ± 8.7 mg/dl.[8]

The concentrations of 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 in the incubation media of both the control and diabetic cotyledons increased progressively during the initial 32 hours of incubation. There was a significant fall in tissue viability from 89% at 32 hours to 64% at 48 hours in the diabetic cotyledons ($p < 0.05$). This decline in tissue viability was associated with a decrease in production of thromboxane and prostacyclin between 32 and 48 hours. There was no significant alteration in tissue viability in the diabetic tissue at any other time period (range, 84% to 92%). Tissue viability remained >80% at all the time periods assessed for the control tissue (range, 86% to 92%).

The concentrations of the stable metabolite of thromboxane, B_2, in the incubation medium during 32 hours of incubation of control and diabetic placental tissue are shown in Fig. 2. The concentrations of B_2 are higher at all sampling times for control tissue than for diabetic tissue. Peak levels after 32 hours were 260.7 ± 44.5 pg/mg in the nondiabetic cotyledons and 173.3 ± 80.5 pg/mg in the diabetic placental tissue. The lower half of the figure shows the B_2 production rates for the sampling time intervals of 0 to 1, 1 to 2, 2 to 4, 4 to 8, 8 to 20, and 20 to 32 hours. The production rates are greatest during the first 8 hours of incubation for both control and diabetic tissue. Thereafter, thromboxane production decreased for both groups, but was higher for control tissue than for diabetic tissue at all time intervals. Average production rates during the 32 hours of incubation were determined by calculation of the slopes of the regression lines between each sampling time interval. In comparison with control tissue, the production rate of thromboxane by diabetic placental cotyledons was significantly less. The average production rate for the nondiabetic placental tissue over the 32-hour period was 7.32 ± 1.37 pg/mg per hour ($n = 7$). The average production rate for the diabetic cotyledons was 5.63 ± 2.81 pg/mg per hour ($n = 5$), and was significantly lower than that of nondiabetic cotyledons (t test, $p < 0.05$). The concentration regression line for thromboxane was also significantly lower for the diabetic group than for the control group (two-way analysis of variance, $p < 0.05$). The ovine placental production rate for thromboxane was similar to that reported for normal human placental tissue.[1]

The placental production rates of prostacyclin for control and diabetic animals, as estimated by measurements of 6-keto-$PGF_{1\alpha}$, are shown in Fig. 3. The concentrations of 6-keto-$PGF_{1\alpha}$ in the incubation medium increased progressively during the 32 hours of study.

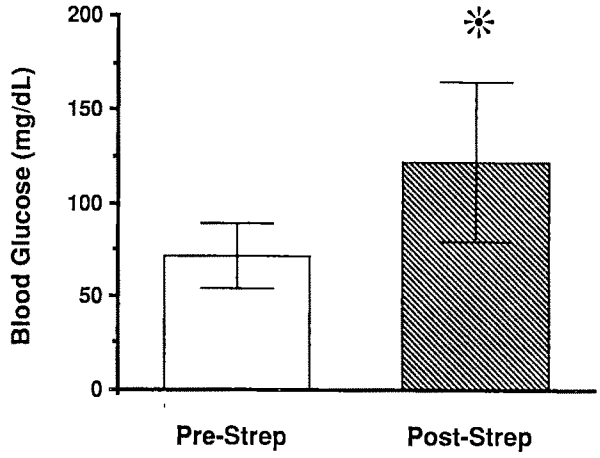


Fig. 1. Maternal fasting venous plasma glucose values before streptozocin and before surgery (71.4 ± 17.0 vs 121.7 ± 43.0 mg/dl, respectively; $n = 6$. Data represent mean ± SE; $^*p < 0.05$).

The concentrations of 6-keto-$PGF_{1\alpha}$ are higher at all sampling times for control tissue than for diabetic tissue. Peak levels after 32 hours were 560.2 ± 147.5 pg/mg for control cotyledonary tissue and 349.3 ± 113.6 pg/mg for diabetic tissue. The lower half of the figure shows the 6-keto-$PGF_{1\alpha}$ production rates for the sampling time intervals of 0 to 1, 1 to 2, 2 to 4, 4 to 8, 8 to 20, and 20 to 32 hours. The production rates are greatest during the first 8 hours of incubation for both control and diabetic tissue. The average production rate over the 32-hour period of incubation was 16.29 ± 4.59 pg/mg per hour ($n = 5$) and 11.44 ± 4.06 pg/mg per hour ($n = 6$) for control and diabetic placental cotyledons, respectively. The production rate of prostacyclin was significantly lower (t test, $p < 0.05$) in diabetic than in control placental tissue. Similarly, the regression line for prostacyclin metabolite concentrations was significantly lower for the diabetic group (two-way analysis of variance, $p < 0.05$). The production rate of prostacyclin in normal ovine cotyledons was approximately two to three times greater than that previously reported for normal human placental tissue in our laboratory.[1,2]

Comment

In this study we used the naturally occurring nitrosourea, streptozocin, to create a large animal model to investigate diabetes in pregnancy. Streptozocin induces destruction of the pancreatic islet cells through an immune-mediated mechanism. In human beings, streptozocin is used therapeutically to treat metastatic pancreatic islet cell carcinoma.[9] This agent has been used previously to induce diabetes in small research laboratory animals such as the rat, dog, and rhesus monkey.[10-12] Spontaneous diabetes mellitus has not been

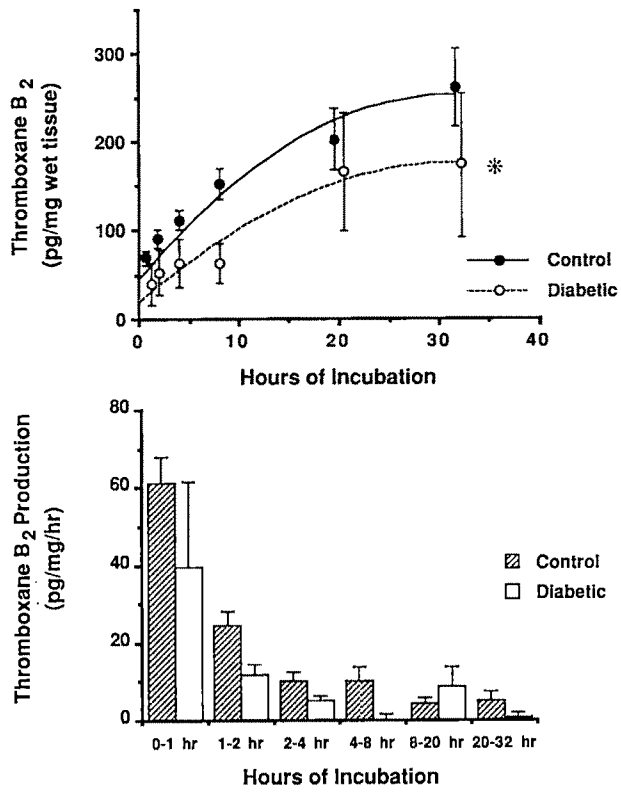


Fig. 2. Production rates of thromboxane by normal and diabetic placental tissues, as estimated by concentrations of its stable metabolite, thromboxane B_2 in incubation medium. Data represent mean ± SE; $*p < 0.05$).

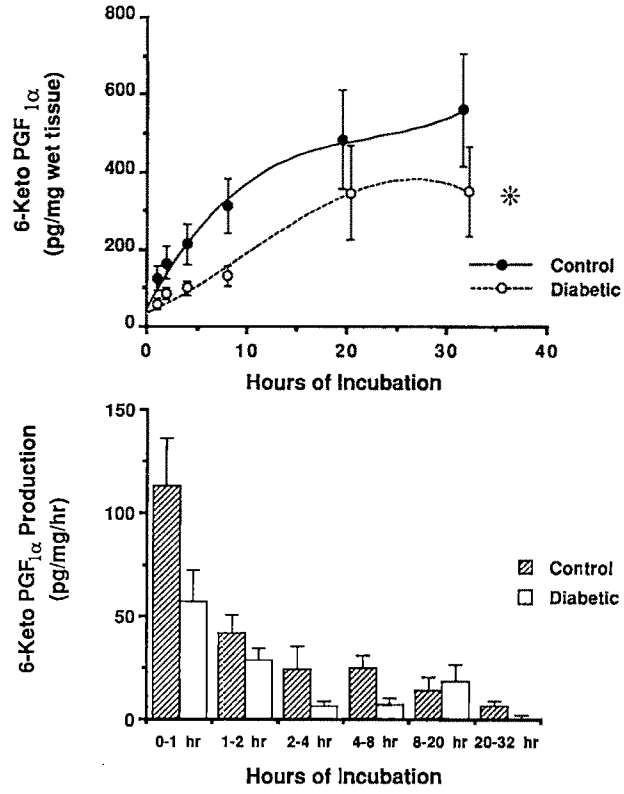


Fig. 3. Production rates of prostacyclin by normal and diabetic placental tissues, as estimated by concentrations of its stable metabolite, 6-keto-$PGF_{1\alpha}$ in incubation medium. Data represent mean ± SE; $*p < 0.05$).

reported in the ovine species and the mean maternal plasma glucose level at late gestation is 63.8 ± 8.7 mg/dl.[8] The observed significant elevation of the fasting plasma glucose level of the streptozocin-treated ewes indicated the induction of a diabetic state. However, the sheep is a ruminant species with little dietary intake of glucose and has a different insulin-glucose response to meals than that of human beings. Our previous work with this animal model showed only small fluctuations in plasma glucose levels (30 to 40 mg/dl) with meals. Thus the mean plasma glucose level is consistently elevated, but unlike the human beings with gestational diabetes, large meal-related glucose fluctuations are not present.

Our study clearly shows that the ovine placenta produces thromboxane and prostacyclin in amounts that approximate human placental production.[1,2] The ovine placenta is epitheliochorial in structure, unlike that of the human being, which is hemochorial in architecture. Despite this fundamental structural difference, the placentas of both species produce the vasoactive eicosanoids thromboxane and prostacyclin at rates that are of a similar order of magnitude, although the ovine placenta produces approximately two to three times more prostacyclin than that of human beings. Our laboratory has previously showed placental thromboxane production rates of 6.3 ± 1.5 pg/mg per hour and placental prostacyclin production rates of 6.7 ± 0.5 pg/mg per hour in normal term human placentas.[1] This results in a ratio of thromboxane to prostacyclin production of 0.94.[2] In contrast, the thromboxane/prostacyclin ratio in the normal near-time ovine placenta is 0.45. This ratio favors the production of prostacyclin. This difference may account in part for the resistance of ovine species to the development of preeclampsia.

In human pregnancy, gestational diabetes is a prominent source of perinatal mortality and morbidity. The effects of the hyperglycemia associated with gestational diabetes on placental function is uncertain. Preliminary investigations in our laboratory with human tissue have suggested a reduction in the placental production rate of the vasodilator prostacyclin in gestational diabetes (Palmer SM, Young S, Walsh SW. Unpublished observations). This reduction was not accompanied by a significant alteration in the placental production rate of the vasoconstrictor thromboxane, so the ratio of thromboxane to prostacyclin favors thromboxane. This is suggestive of a possible mechanism for reduced placental blood flow in gestational diabetes.

We observed a significant reduction in tissue viability and eicosanoid production at 48 hours of incubation

in the ovine diabetic cotyledons. High glucose levels may have directly impaired eicosanoid synthesis or affected viability. Rakoczi et al.[6] showed a dose-dependent inhibition of human placental prostacyclin synthesis by glucose. Another possibility is that hyperglycemia may have decreased cellular proliferation of the placenta. An adverse effect of hyperglycemia on in vitro cellular proliferation of human placental tissue has been reported.[13] A reduction of in vivo cellular proliferation resulting from hyperglycemia is a possible mechanism to account for the reduced in vitro eicosanoid production and impaired tissue viability observed after 48 hours of incubation. Consistent with these findings, we anecdotally noted a decrease in the tissue turgor and quality of the placental cotyledons obtained from the diabetic animals. There was no significant alteration in tissue viability at any other time period. Thus an alteration in tissue viability is not the cause of the observed changes in eicosanoid production.

The placental production of prostacyclin in the diabetic ewe was significantly reduced when compared with the nondiabetic control animals. This is consistent with earlier observations in the human gestational diabetic (Palmer SM, Young S, Walsh SW. Unpublished observations). The placenta of the nondiabetic ewe produced 1.4 times as much prostacyclin as the diabetic placenta. However, unlike human gestational diabetes, we found a significant reduction in thromboxane production in the ovine diabetic placenta. The control ovine placenta produced 1.3 times as much thromboxane as the diabetic placenta. The ovine diabetic placental thromboxane/prostacyclin ratio was 0.49. Thus the thromboxane/prostacyclin ratio in diabetic animals was not altered, despite the reduction in the production rate of these vasoactive eicosanoids ($p > 0.5$).

The reason for the observed differences between the ovine and human placental eicosanoid production rates in gestational diabetes may relate to interspecies variation in placental structure and function. Another potential explanation for the observed differences in production of thromboxane and prostacyclin is a difference in the availability or metabolism of arachidonic acid in the diabetic animals. Alterations in cholesterol metabolism are frequently associated with the alterations of glucose metabolism in diabetes mellitus in human beings. The diet of the ewe is deficient in lipids, potentially resulting in substrate reduction for cyclooxygenase metabolism. It is also possible that the streptozocin directly altered placental function because the agent was administered during pregnancy. It is believed that this compound has a specific affinity for the pancreatic islet cell.[10] However, to be certain that streptozocin has no direct effect on placental function, the agent would need to be administered before conception.

We conclude that the ovine placenta produces thromboxane and prostacyclin. The placental production rates of these eicosanoids in human and ovine placentas are of an equivalent order of magnitude. It is of interest that the ratio of thromboxane/prostacyclin is different between the two species. In human beings approximately equal amounts of thromboxane and prostacyclin are produced, whereas in the sheep prostacyclin is produced at a rate that is over twice that of thromboxane. The epitheliochorial ovine placenta possesses a distinct maternal vascular component, whereas in the human hemochorial placenta there is only a fetal vascular component. Therefore the ovine placenta contains more vascular tissue capable of producing prostacyclin, possibly accounting for the observed differences in production. The presence of maternal hyperglycemia in the ewe is associated with a reduction in the placental production of both thromboxane and prostacyclin, without alteration in the thromboxane/prostacyclin ratio.

We would like to acknowledge Wil Morgan and the Upjohn Company for their assistance in this research.

REFERENCES

1. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol 1985;152:335-40.
2. Walsh SW, Behr MJ, Allen NH. Placental prostacyclin production in normal and toxemic pregnancies. Am J Obstet Gynecol 1985;151:110-5.
3. Davis TME, Mitchell MD, Turner RC. Prostacyclin and thromboxane metabolites in diabetes. Lancet 1979;2:789-90.
4. Davis TME, Bown E, Finch DR, et al. In vitro venous prostacyclin production, plasma 6-keto-prostaglandin $F_{1\alpha}$ concentrations, and diabetic retinopathy. Br Med J 1981; 282:1259-62.
5. Halushka PV, Rogers RC, Loahold CB, et al. Increased platelet thromboxane synthesis in diabetes mellitus. J Lab Clin Med 1981;97:87-96.
6. Rakoczi I, Tihanyi I, Gero Gy, et al. Release of prostacyclin (PGI_2) from trophoblast in tissue culture: the effect of glucose concentration. Acta Physiol Hung 1988;71(4): 545-9.
7. Dudak C, Kefalides A, Sinzinger H, et al. Reduced umbilical artery prostacyclin formation in complicated pregnancies. Am J Obstet Gynecol 1982;144:792-5.
8. Hay WW, Sparks JW, Wilkening RB, et al. Partition of maternal glucose production between conceptus and maternal tissues in sheep. Am J Physiol 1983;245:E347-50.
9. DeVita VT. Principles of cancer therapy. In: Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS, eds. Harrison's principles of internal medicine. 11th ed. New York: McGraw-Hill, 1987:443.
10. Rakieten N, Rakieten ML, Naddkarni MV. Studies on the diabetogenic action of streptozotocin. Cancer Chemother Rep 1963;29:91-8.
11. Junod A, Lambert AE, Orci L, et al. Studies of the diabetogenic action of streptozotocin. Proc Soc Exp Biol Med 1963;126:201-5.
12. Pitkin RM, Reynolds WA. Diabetogenic effects of streptozotocin in rhesus monkeys. Diabetes 1970;19:85-90.
13. Nelson DM, Curran EM. High glucose levels decrease proliferation of cultured human fetal cells from placenta. Am J Obstet Gynecol 1989;161:1553-8.

Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns

Naguib A. Samaan, MD, PhD, Pamela N. Schultz, RN, MS, and Frank K. Pham, BS
Houston, Texas

We measured insulin-like growth factor II and nonsuppressible insulin-like activity levels in the sera of newborn infants with different birth weights and gestational ages to determine the significance of these peptides in fetal growth. Our results obtained by use of one-way analysis of variance showed that the insulin-like growth factor II and nonsuppressible insulin-like activity levels in premature, average-weight-for-gestational-age, large-for-gestational-age, and small-for-gestational-age newborns were significantly different ($p < 0.01$). Although levels in the premature neonates were less than the other three groups and large-for-gestational-age neonates had a higher insulin-like growth factor II level than the other three groups, maternal insulin-like growth factor II levels in all groups were similar. These results suggest that insulin-like growth factor II may play a major role in fetal growth. (AM J OBSTET GYNECOL 1990;163:1836-9.)

Key words: Insulin-like growth factor, nonsuppressible insulin-like activity, fetal growth

The mechanism responsible for fetal growth in utero remains to be determined. The description of the sequence of the insulin-like growth factors I and II, which were isolated from the nonsuppressible insulin-like activity by Rinderknecht and Humbel[1] in 1976, resulted in marked progress in this field. The isolation of these growth factors, also known as somatomedins, was preceded by the description of "typical" and "atypical" insulin-like activity by Samaan et al.[2, 3] that was later termed "suppressible" and "nonsuppressible" insulin-like activity by Froesch et al.[4] The serum component (sulfation factor), which mediated the growth hormone-induced incorporation of sulfate into cartilage, was described by Salmon and Daughaday.[5]

In addition to purifying insulin-like growth factors I and II from nonsuppressible insulin-like growth factors, Rinderknecht and Humbel[1] also determined their amino acid sequence, which was found to be similar to proinsulin. Zapf et al.[6] found that both insulin-like growth factors I and II fulfill all the criteria of somatomedins. The origin of somatomedins has been a subject of extensive research. Samaan et al.[7, 8] showed strong evidence that atypical or nonsuppressible insulin-like activity was formed mainly in the liver because they found that the atypical insulin-like activity level was significantly lower in patients with liver cirrhosis and was lowered further after portocaval shunt. They also showed that infusion of regular insulin into the portal circulations of dogs resulted in the increase of the level of the atypical or nonsuppressible insulin-like activity.[9] These observations have been confirmed by various investigators.[6, 10-12] Recently, Daughaday et al.[13] also showed evidence that the liver plays a major part in the formation of sulfation factor when growth hormone was infused in the liver and that insulin has synergetic effect on growth hormone. It was then concluded that growth hormone, insulin, and nutritional factors are most responsible for the formation of the somatomedins.[14]

Underwood et al.[15] purified and separated somatomedin C, which was found to be structurally similar to insulin-like growth factor I and has potent action in cartilage similar to the sulfation factor. Conversely, insulin-like growth factor II was found to be more potent on adipose tissue.[6, 14, 15]

Insulin-like growth factor I has been measured in pregnant mothers and umbilical cords, and it was found that growth hormone and insulin-like growth factor I do not appear related to the growth of the fetus.[14, 16] However, the relationship of insulin-like growth factor II to fetal growth and time of gestation has not been clarified.

This study was undertaken to determine both the insulin-like growth factor II levels, as measured by the radioimmunoassay, and the nonsuppressible insulin-like activity level, as measured by the fat pad assay in the sera of the newborn infants with different birth weights, gestational ages, and 1-minute Apgar scores

From the Section of Endocrinology, Department of Medical Specialties, The University of Texas M.D. Anderson Cancer Center.
Supported by the Nancy D. Carmichael Estate for Cancer Research and the Robert V. Davidson Estate for Cancer Research, Houston, Texas.
Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Naguib A. Samaan, MD, PhD, Section of Endocrinology, The University of Texas M.D. Anderson Cancer Center, Box 15, 1515 Holcombe Blvd., Houston, TX 77030.
6/6/24781

so that the significance of these peptides in fetal growth could be determined.

Material and methods

At the time of delivery, sera were collected by venipuncture from 54 women with normal pregnancies at term (37 to 41 weeks) and who were delivered of average-for-gestational-age (AGA) newborns, 53 women who were at term and were delivered of large-for-gestational-age (LGA) infants, five women who were delivered of small-for-gestational-age (SGA) infants, and eight women who were delivered of premature newborns. Categories were based on data provided by Williams et al.[17] in a study conducted in California of 2,288,806 live births. Infants who had reached 37 weeks' gestation and whose weight was ≤10th percentile were considered SGA. Those with weights ≥90th percentile were considered LGA, and those with weights between the 11th and 89th percentiles were considered AGA. An infant was considered premature if the gestational age at birth was ≤36 weeks. The premature newborns in this study had average weights for their gestational age.

Umbilical cord blood was collected from the placental side at the time of delivery immediately after the cord was clamped. The blood was collected in nonheparinized tubes. The serum was separated and kept at −20° C until the time of assay. This study was done with the approval of the Human Protection Committee and informed consent was obtained from each mother.

The insulin-like growth factor II was measured by the radioimmunoassay method after separation of insulin-like growth factor II from the binding proteins by use of the C18 Sep-pak silica cartridge chromatography and a vacuum elution system method. The insulin-like growth factor II was measured by the double-antibody radioimmunoassay method.[18] The first antibody was a monoclonal antibody raised against rat insulin-like growth factor II obtained from Enzyme International (Troy, Va.). It had a 100% crossreactivity with human insulin-like growth factor II and rat insulin-like growth factor II; human insulin-like growth factor I was 10% and bovine insulin was <0.01%. Biosynthetic human insulin-like growth factor II (Eli Lilly & Co., Indianapolis) was used for iodination and assay standards. Interassay variation in insulin-like growth factor II was <20%.

The nonsuppressible insulin-like activity was measured in the serum with the fat pad assay in the presence of glucose carbon 14 isotope and insulin antibody as described by Samaan et al.[3] To make an overall comparison that describes significant differences between groups, a one-way analysis of variance that uses the F test was done to determine the effect of birth weight and gestation on nonsuppressible insulin-like activity and insulin-like growth factor II. Specific differences were analyzed by means of the Tukey test.

Table I. Nonsuppressible insulin-like activity and insulin-like growth factor II levels in premature, SGA, AGA, and LGA newborns

Newborns	*Nonsuppressible insulin-like activity (μU/ml)*	*Insulin-like growth factor II (ng/ml)*
Premature (n = 10)	125 ± 59	48.2 ± 10.9
SGA (n = 4)	211 ± 83	54.0 ± 6.6
AGA (n = 54)	153 ± 121	57.9 ± 11.1
LGA (n = 53)	272 ± 161	63.8 ± 11.4
F test	4.50 ($p < 0.01$)	6.01 ($p < 0.01$)

The one-way analysis of variance that uses the F test was done to determine the effect of birth weight and gestation.

Results

Table I summarizes the mean ± SD of the nonsuppressible insulin-like activity and insulin-like growth factor II levels of the premature neonates, SGA, AGA, and LGA neonates. When analysis of variance was applied to the nonsuppressible insulin-like activity levels, there was a significant difference found among the four groups ($F = 4.50$, $p < 0.01$). The nonsuppressible insulin-like activity levels in the premature neonates was less than in the other three groups, and the nonsuppressible insulin-like activity levels in the LGA group was greater than in the other three groups. The significant differences according to the Tukey test was LGA versus premature ($q = 4.57$, $p < 0.01$) and LGA versus AGA ($q = 4.49$, $p < 0.01$). The insulin-like growth factor II level was similar to the nonsuppressible insulin-like activity in that the insulin-like growth factor II levels of the four groups were significantly different ($F = 6.01$, $p < 0.01$). The insulin-like growth factor II level of the premature neonate was less than the other three groups, and the LGA neonates had higher insulin-like growth factor II levels than in the other three groups. The significant difference according to the Tukey test was LGA versus premature ($q = 5.13$, $p < 0.05$).

The maternal insulin-like growth factor II levels in all patients studied were similar and not significantly different from those found in nonpregnant women of the same age range. The mean ± SD of insulin-like growth factor II levels in the mothers was significantly higher than in all groups of neonates (89.1 ± 5.9, $p < 0.001$). There was no correlation found between maternal weight and their newborn birth weights.

Comment

Little is known about the regulation of somatomedin production in the fetus.[14] It has been shown that

somatomedins do not pass through the placental barrier.[14] The source of the embryonic somatomedins appears to be the fetus itself. Nutritional factors have been proposed to play a primary role in the regulation of somatomedin in the fetus.[14] Both insulin-like growth factor I and II levels are formed in the liver, but to a lesser extent in different tissues including the placenta.[7, 14, 19, 20] It has also been shown that there is a decrease in insulin-like growth factors after experimental manipulation designed to produce growth retardation in the fetal rat.[20, 21]

Brinsmead and Liggins[22] reported that growth hormone is not required for fetal growth because neither somatomedin levels nor growth are affected by hypophysectomy in lambs or decapitation in the uterus. Furthermore, the anencephalic fetuses grow normally during intrauterine life. We and others have also shown that the insulin-like growth factor I level is significantly lower in infants than in adults[16] and was significantly lower in premature newborns than in AGA infants; however, there was no significant difference between the LGA infants and the AGA infants, and we speculated that other growth factors may play a part.

Both insulin-like growth factor I and II levels are found in the circulation with carrier proteins. The exact role of somatomedin-binding proteins in the circulation and how they act to deliver somatomedins to their target tissues is poorly understood. One well-established function is to increase the plasma half-life of somatomedin from 8 to 30 minutes when not protein bound to 3 to 6 hours in the presence of binding proteins.[19] Another apparent role is to provide a reservoir for somatomedin because serum is its primary storage site. It appears that the peptides, when protein bound, exert little of their potential activity. Although this does not necessarily indicate that the naturally occurring high-molecular mass complexes are devoid of activity, evidence exists to suggest that a portion of the total nonsuppressible insulin-like activity level in serum exerts its biologic action when protein bound.[14, 23]

It has also been shown that the binding proteins may interfere with the radioimmunoassay of the somatomedins and acid ethanol extraction was introduced by Daughaday et al.[18] and the use of BioGel P-60 column chromatography appears to be the method of choice since total binding proteins are removed. The combined use of a radioimmunoassay and biologic assay seems to be the best approach for measurements of somatomedins. The fat pad assay appears to measure both insulin-like growth factor I and II levels as well as other related undiscovered peptides.[6]

Our findings indicate that although the insulin-like growth factor II level is low in premature infants, it is also significantly higher in the LGA infants than in the AGA infants. Similar results were obtained by measuring the nonsuppressible insulin-like activity, which is predominantly insulin-like growth factor II.[6, 14] The disparity concerning the rank order of nonsuppressible insulin-like activity levels may be a result of the small number of cases with SGA. The differences in the levels of nonsuppressible insulin-like activity and insulin-like growth factor II in the four categories may reflect a difference in gestational age, birth weight, or both. Thus it appears that insulin-like growth factor II may play a major role in fetal growth.

REFERENCES

1. Rinderknecht E, Humbel RE. Amino-terminal sequences of two polypeptides from human serum with nonsuppressible insulin-like activity using rat adipose tissue. I. A proposed procedure. Proc Natl Acad Sci USA 1976;73:4379-81.
2. Samaan NA, Dempster WJ, Fraser R, Stillman D. Immunological studies on two forms of circulating insulin. Biochem J 1962;82:29.
3. Samaan NA, Dempster WJ, Fraser R, Please NW, Stillman D. Further immunological studies on the form of circulating insulin. J Endocrinol 1953;24:263-77.
4. Froesch ER, Burgi H, Ramseier EV, Bally P, Labhart A. Antibody suppressible and nonsuppressible insulin-like activities in human serum and their physiological significance. An insulin assay with adipose tissue of increased precision and specificity. J Clin Invest 1963;42:1816-36.
5. Salmon WD Jr, Daughaday WH. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J Lab Clin Med 1957;49:825-36.
6. Zapf J, Rinderknecht E, Humbel RE, Froesch ER. Nonsuppressible insulin-like activity (NSILA) from human serum: recent accomplishments and their physiologic implications. Metabolism 1978;27:1803-28.
7. Samaan NA, Stillman D, Fraser R. Abnormalities of serum insulin-like activity in liver disease. Lancet 1962;2:1287-90.
8. Samaan NA, Fraser R, Dempster WJ. Typical and atypical forms of serum insulin. Diabetes 1963;12:339-47.
9. Samaan NA, Dempster WJ, Fraser R, Stillman D. Changes in levels of "atypical" circulating insulin after infusing "typical" insulin through the liver. J Clin Endocrinol 1963;26:1-10.
10. Wu A, Grant DB, Hambley J, Levi AJ. Reduced serum somatomedin activity in patient with chronic liver disease. Clin Sci Mol Med 1974;47:359-66.
11. Schlumpf J, Heimann R, Zapf J, Froesch ER. Nonsuppressible insulin-like activity and sulphation activity in serum extracts of normal subjects, acromegalic and pituitary dwarfs. Acta Endocrinol (Copenh) 1976;81:28-42.
12. Takano K, Hizuka N, Shizume K, Hayashi N, Motoike Y, Obata H. Serum somatomedin peptides measured by somatomedin A radioreceptor assay in chronic liver disease. J Clin Endocrinol Metab 1977;45:828-32.
13. Daughaday WH, Phillips LS, Mueller MC. The effect of insulin and growth hormone on the release of somatomedin by the isolated rat liver. Endocrinology 1976;98:1214-9.
14. Hall K, Sara VR. Growth and somatomedins. In: Vitamins and hormones. New York: Academic Press, 1983:40.
15. Underwood LE, Hintz RL, Voina SJ, Van Wyk JJ. Human somatomedin, the growth hormone-dependent sulfation factor, is anti-lipolytic. J Clin Endocrinol Metab 1972;35:194-8.
16. Samaan NA, Schultz PN, Johnston DA, Creasy RW, Gonik B. Growth hormone, somatomedin C, and insulin-like activity levels compared in premature, small, average birth

weight, and large infants. AM J OBSTET GYNECOL 1987; 157:1524-8.
17. Williams RL, Creasy RK, Cunningham GC, Hawes WE, Norris RD, Tashiro M. Fetal growth and perinatal viability in California. Obstet Gynecol 1982;59:624-32.
18. Daughaday WH, Parker KA, Borowsky S, Trivedi B, Kapadia M. Measurement of somatomedin-related peptides in fetal, neonatal, and maternal rat serum by insulin-like growth factor (IGF)-I radioimmunoassay, IGF-II radioreceptor assay (RRA), and multiplication-stimulating activity RRA after acid-ethanol extraction. Endocrinology 1982;110:575-81.
19. Baxter RC. The somatomedins: insulin-like growth factors. In: Advances in clinical chemistry. New York: Academic Press, 1986:25.
20. Chard T. Hormonal control of growth in the human fetus. J Endocrinol 1989;123:3-9.
21. Hill DJ, Fekete M, Milner RDG, DePrins F, VanAssche A. Reduced plasma somatomedin activity during experimental growth retardation in the fetal and neonatal rat. In: Spencer EM, ed. Insulin-like growth factors/somatomedins. Basic chemistry, biology and clinical importance. Berlin: Walter deGruyter, 1983:345.
22. Brinsmead MW, Liggins GC. Serum somatomedin activity after hypophysectomy and during parturition in fetal lambs. Endocrinology 1979;105:297.
23. Poffenbarger PL. Nonsuppressible insulin-like proteins in health and disease. Spec Topics Endocrinol Metab 19;1: 109-40.

Growth factor activity in the blood of women in whom preeclampsia develops is elevated from early pregnancy

Robert N. Taylor, MD, PhD,[a] David C. Heilbron, PhD,[b] and James M. Roberts, MD[a]
San Francisco, California

Preeclampsia is a pregnancy-specific disorder of uncertain cause and pathophysiology that appears to be associated with endothelial cell injury. Our current studies demonstrate that a pregnancy growth factor activity is elevated compared with postpartum values in the blood of women with preeclampsia months before the onset of clinical manifestations of toxemia. A cohort of primigravid women was followed throughout pregnancy and multiple serial plasma samples from six women with preeclampsia and six matched normal women were assayed for mitogenic activity. The data indicated that the ratio of predelivery/postdelivery plasma mitogenic activity was greater in women predestined to meet strict criteria for the diagnosis of preeclampsia compared with matched normal primigravid women. Growth factor activity could distinguish women in whom preeclampsia would develop from their normal peers throughout pregnancy, and as early as the first trimester of gestation ($p < 0.05$). Similar studies performed with plasma obtained >6 weeks post partum, when the two groups of patients were clinically indistinguishable, revealed no differences in this index of mitogenic activity. Our results indicate that elevated mitogenic activity ratios of prepartum versus postpartum plasma antedate the clinical recognition of preeclampsia, and return to normal with the resolution of the syndrome. (AM J OBSTET GYNECOL 1990;163:1839-44.)

Key words: Preeclampsia, plasma growth factors

From the Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences,[a] and the Department of Epidemiology and Biostatistics,[b] University of California, San Francisco.
Supported by National Institutes of Health grants HD 28410 and HD 22873.
Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
This is paper no. 8 from the University of California, San Francisco, Preeclampsia Program Project.
Reprint requests: Robert N. Taylor, MD, PhD, W. M. Keck Laboratory of Reproductive Molecular and Developmental Biology, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, School of Medicine, M-1489, San Francisco, CA 94143-0132.
6/6/24773

Preeclampsia is a pregnancy-specific disorder of uncertain cause and pathophysiology. Particular aspects of the pathologic changes associated with the syndrome of preeclampsia suggest that generalized maternal vascular endothelial cell dysfunction could explain the vasospasm, edema, proteinuria, coagulopathy, and renal and hepatic abnormalities that are hallmarks of this disease.[1] A variety of clinical observations lends credence to the hypothesis of endothelial cell injury in this syndrome. The pathognomonic renal lesion found in women with preeclampsia, glomeruloendotheliosis,[2] provides morphologic evidence of maternal endothelial cell perturbation in this condition, whereas elevations

of plasma fibronectin[3] and factor VIII antigen[4] and decreased levels of prostacyclin[5] reflect biochemical dysfunction in these cells. Finally, the increased rate of disappearance of circulating Evans blue dye[6] suggests that the functional integrity of maternal endothelia is altered in preeclampsia. That these changes might be caused by a circulating factor is suggested by our finding that predelivery sera from women with preeclampsia injure human endothelial cells in vitro.[7] We have also found that mitogenic activity is increased in prepartum blood obtained from these patients.[8] We hypothesized that increased growth factor activity was a result of the direct release of mitogens from injured endothelial cells or platelet activation by perturbed endothelium.[9] Our previous studies demonstrated the presence of this activity in women with overt preeclampsia. This study was designed to determine whether increased mitogenic activity antedated the clinical syndrome of preeclampsia.

Material and methods

Patient selection. All pregnant women who registered for prenatal care at the affiliated hospitals of the University of California, San Francisco, were invited to participate in a prospective study of preeclampsia. Among those who entered the study in 1988, a cohort of 12 primigravid women (six normal and six preeclamptic singleton pregnancies; see below) with complete clinical and biochemical data were studied. All participating patients gave written informed consent to donate serial plasma samples for the purpose of the study. The blood samples were obtained at the times of routine clinical testing, starting no later than the first trimester of pregnancy. Plasma was prepared within 12 hours of venipuncture, and aliquots were stored at −70° C. In this study patients had from four to eight plasma samples (mean, 6) obtained throughout pregnancy, with each trimester represented. Thus the quantification of growth factor activity in these pregnancies represents the analysis of 65 individual plasma samples. Demographic and clinical data were collected at routine obstetric visits. All patients were followed throughout the pregnancy until at least 6 weeks post partum.

A panel of five obstetricians reviewed the clinical course of each patient and a post hoc assignment was made to normal or preeclampsia groups. Rigid criteria, as recommended by Chesley,[10] were established for the diagnosis of preeclampsia. Patients with preeclampsia were defined as follows: nulliparous, not known to be hypertensive before pregnancy, manifesting an increase in systolic pressure of 30 torr or diastolic pressure of 15 torr (or an increase in mean arterial blood pressure [MAP] >20 torr) compared with blood pressures obtained before 20 gestational weeks, proteinuria ≥0.5 gm/24 hours or ≥30 mg/dl by dipstick of catheterized urine, serum uric acid level >5.5 mg/dl, return to normal blood pressure and clearance of proteinuria by 12 weeks post partum. Normal, control patients were matched with those with preeclampsia for race and gestational age at delivery. Patients with evidence of illicit drug or cigarette use, chronic hypertension, or any other medical diseases were excluded.

Growth factor (mitogenesis) bioassay. Quiescent cultures of human foreskin fibroblasts were used in the growth factor bioassay optimized and described previously.[8] A total of 65 aliquots of frozen plasma were gently thawed at room temperature and diluted to a final concentration of 2% in Dulbecco's modified Eagle's medium, supplemented with Hepes (20 mmol/L), bovine serum albumin (500 μg/ml), transferrin (5 μg/ml), insulin (1 μg/ml), and heparin (2 U/ml). Samples were never subjected to more than one thaw. The plasma-reconstituted media were added to triplicate wells of cultured fibroblasts. Incorporation of tritiated thymidine into deoxyribonucleic acid of these cells was determined after precipitation of the nucleic acids with 10% trichloroacetic acid, and liquid scintillation counting of the resuspended precipitate was performed. To correct for individual variation in the growth factor analyses, the results of each plasma sample mitogenic activity are expressed as a multiple of the same patient's immediate postpartum sample, as described previously.[8] In the aforementioned study, Musci et al.[8] demonstrated that differences between the mitogenic activities of preeclamptic and normal blood samples were present in predelivery but not postpartum specimens. Thus normalization to the immediate postpartum growth factor activity of each patient resulted in a "mitogenic index" to be compared among all patients. Furthermore, to minimize bias in the bioassays, sets of samples from normal patients and those with preeclampsia were always measured simultaneously within the same fibroblast cultures. Average mitogenic indices for each trimester of pregnancy were calculated from the pooled results of samples obtained from each patient during that trimester.

Statistical analysis and interpretation. Study results are presented as the mean values (±SD) of each clinically defined group. The results of blood pressure determination and analyses of proteinuria throughout the course of pregnancy were collected from the patients' prenatal records. The MAP for each patient at term was derived from the average of at least four independent readings during the last week of pregnancy, according to the calculation: MAP = Diastolic + ([Systolic − Diastolic]/3). Average MAP and urine protein concentration determinations were quantified for each patient in each trimester of pregnancy. Comparisons between the two study groups (12 subjects in total) were made with the nonparametric

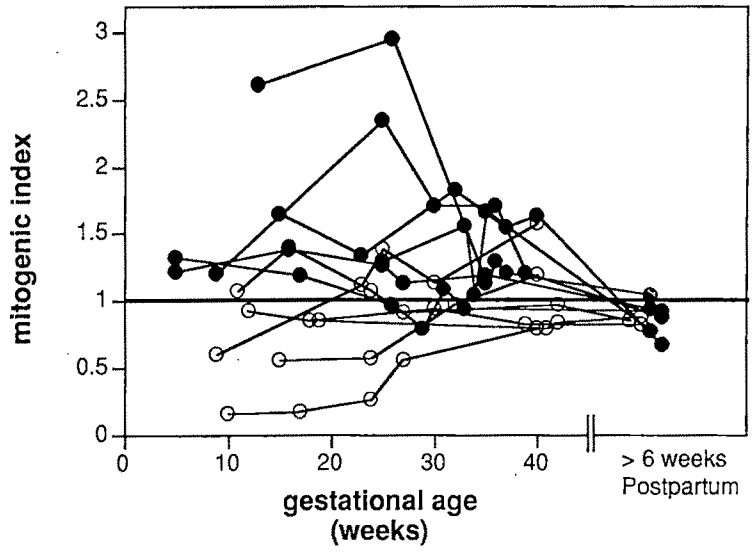


Fig. 1. A total of 65 plasma samples collected longitudinally throughout pregnancy from 12 patients were assayed for mitogenic activity as described in Material and methods. Mitogenic indices were computed and normalized by each patient's immediate postpartum sample as described. The indices are plotted against the time of blood sampling (weeks of gestation from last menses). Patients who ultimately met strict criteria for the diagnosis of preeclampsia are represented with *closed circles*, whereas matched normal pregnant women are indicated by *open symbols*. The mitogenic indices of each individual patient are connected by lines. Postpartum plasma samples were obtained at >6 weeks after delivery.

Table I. Patient characteristics and intrapartum clinical data

	Preeclamptic ($n = 6$)	*Normal* ($n = 6$)	p*
Maternal age at delivery (yr)	25 ± 5†	30 ± 5	NS
Parity	0	0	NS
Infant weight at delivery (gm)	2507 ± 884	3277 ± 453	NS
Gestational age at delivery (wk from last menses)	37 ± 5	39 ± 2	NS
Hematocrit (%, vol/vol)	37 ± 2	37 ± 5	NS
Serum creatinine (mg/dl)	0.7 ± 0.2	0.6 ± 0.2	NS
Mean arterial pressure (torr)	108 ± 7	80 ± 7	<0.01
Proteinuria (mg/dl)‡	84 ± 29	0	<0.01
Serum uric acid (mg/dl)	6.4 ± 0.5	4.0 ± 0.7	<0.02

*Level of significance by Mann-Whitney test.

†Mean ± SD.

‡Semiquantitative dipstick analysis of catheterized urine samples.

Mann-Whitney test; values of $p < 0.05$ were considered to reflect significant differences.

Results

The clinical and biochemical findings of the patients at term are shown in Table I. As anticipated by the diagnostic criteria used to identify women as having preeclampsia, this group of parturients had elevations in mean arterial blood pressure and proteinuria ($p < 0.01$) and serum uric acid concentrations ($p < 0.02$) compared with matched normal controls. In addition, trends toward lower maternal age and decreased infant birth weight were noted in the patients with preeclampsia (Table I).

The longitudinal patterns of growth factor activity, as determined by the mitogenesis bioassay, demonstrated significant differences between pregnancies that were ultimately identified as normal and preeclamptic. The data presented in Fig. 1 demonstrate the mitogenic indices determined throughout pregnancy for each of the 12 study patients. The majority of women who ultimately met strict criteria for the diagnosis of preeclampsia had prepartum plasma mitogenic indices >1. By contrast, mitogenic indices for matched normal

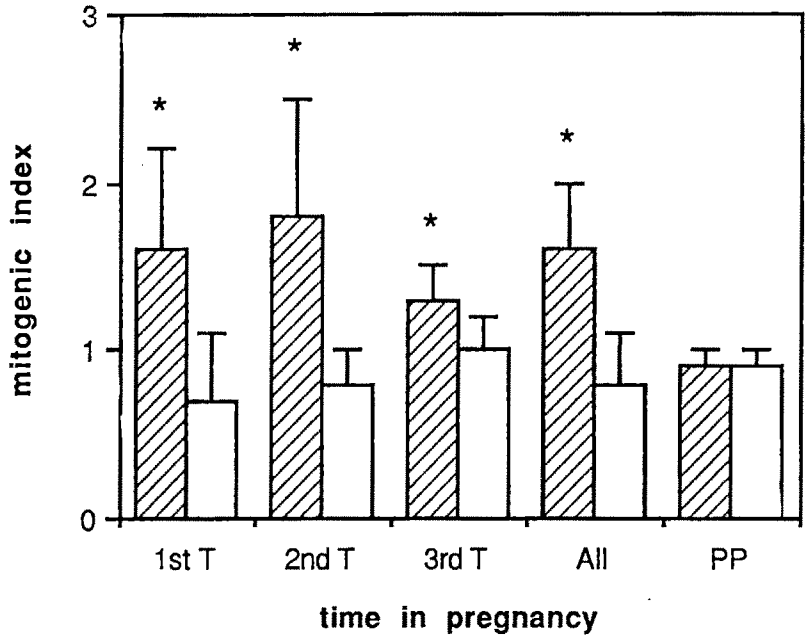


Fig. 2. Mitogenic indices (predelivery/postdelivery counts per minute incorporated per well of human fibroblasts) of prospectively collected plasma were quantified throughout pregnancy and postpartum by use of tritiated thymidine incorporation assays as described in Material and methods. The mean (±SD) mitogenic indices averaged over each of the three trimesters *(T)*, the entire pregnancy *(All)*, and at >6 weeks post partum *(PP)* are shown. Patients ultimately diagnosed with preeclampsia are represented by the *hatched bars*, whereas the matched normal parturients are represented by *open bars*. Histograms indicate data from all 12 patients in the second, third, and *All* trimester categories. Data from four patients in each group are shown in the first trimester, and six women with preeclampsia and three normal patients are represented in the >6 weeks postpartum data. *Asterisk* indicates values that are different from those of normal parturients by Mann-Whitney tests ($p < 0.05$).

Table II. Longitudinal assessment of blood pressure, proteinuria, and growth factor activity during pregnancy

	First trimester	*Second trimester*	*Third trimester*
Mean arterial pressure (torr)			
Preeclamptic	81 ± 8	88 ± 7	101 ± 7
Normal	78 ± 8	77 ± 5	78 ± 5
p*	NS	<0.05	<0.01
Proteinuria (mg/dl)			
Preeclamptic	0	0	48 ± 29
Normal	0	0	0
p*	NS	NS	<0.01
Mitogenic index (ratio)			
Preeclamptic	1.6 ± 0.6	1.8 ± 0.7	1.3 ± 0.2
Normal	0.7 ± 0.4	0.8 ± 0.2	1.0 ± 0.2
p*	<0.05	<0.01	<0.02

*Level of significance by Mann-Whitney test.

women were generally <1. By this index growth factor activity appeared greatest in plasma collected during the first two trimesters of pregnancy from women in whom preeclampsia ultimately developed (Fig. 1).

To statistically evaluate these data, we calculated the average mitogenic index for each patient during the entire pregnancy, independently for each of the three trimesters, and during a late postpartum period (>6 weeks from delivery). Thus no patient was represented more than once in a given gestational period, even if multiple plasma samples were tested within a single trimester. Because the distribution of the average mitogenic index for each subject was not known to be normally distributed, analysis by the nonparametric Mann-Whitney test was selected. Women predestined to meet criteria for the diagnosis of preeclampsia had significantly elevated mitogenic indices throughout pregnancy compared with matched normal primigravid women (1.6 ± 0.4 vs 0.8 ± 0.3, mean ± SD, $p < 0.01$; see Fig. 2). Similar statistical analyses of growth factor activity for each trimester of pregnancy distinguished women in whom preeclampsia developed from their normal peers in each trimester (first trimester, 1.6 ± 0.6 vs 0.7 ± 0.4, $p < 0.05$; second trimester, 1.8 ± 0.7 vs 0.8 ± 0.2, $p < 0.01$; third trimester, 1.3 ± 0.2 vs 1.0 ± 0.2, $p < 0.02$; Fig. 2). The results of the third-trimester analyses confirmed our initial report of mitogenic indices in preeclamptic and normal pregnancy sera at term (1.4 ± 0.2 vs 1.1 ± 0.2, $n = 29$, $p < 0.01$).[8] Despite significant differences between the

normal and preeclamptic groups before delivery, however, by 6 weeks post partum both groups had indistinguishable mitogenic indices (0.9 ± 0.1 vs 0.9 ± 0.1; Fig. 2).

Longitudinal analyses of mean arterial blood pressures and proteinuria in the same patients are presented in Table II. A statistically significant difference between the average MAP of the preeclamptic and normal groups was demonstrable by the second trimester of pregnancy (88 ± 7 vs 77 ± 5 torr, $p < 0.05$). The MAP values in these groups corresponded to average blood pressures of 125/74 and 106/63 torr in women with preeclampsia and normal patients, respectively. However, the elevation of MAP in the preeclamptic group only achieved a diagnostically significant level during the third trimester of pregnancy (101 ± 7 vs 78 ± 5 torr, $p < 0.01$), reflecting average blood pressures of 144/81 and 112/64 torr. Differences in proteinuria between the two groups were noted only in the third trimester (Table II).

Comment

We used strict diagnostic criteria in patient selection, analyses of multiple, longitudinally collected samples from the same cohort of patients, and calculation of a plasma mitogenic index by normalizing to each woman's immediate postdelivery plasma growth factor activity. As shown in Fig. 1, this strategy resulted in the resolution of 65 individual plasma samples into two clusters with minimal overlap that corresponded to pregnancy outcome despite the limited number (12) of study subjects. Moreover, when the data were combined according to trimester of pregnancy, conservative, nonparametric statistical analyses demonstrated that the plasma mitogenic indices in normal and preeclamptic pregnancies differed throughout gestation (Fig. 2).

The results of this study confirm our earlier finding[8] that the mitogenic index is elevated in the blood of women with the classic syndrome of preeclampsia in relation to appropriately matched, normal controls. In addition, this study revealed that elevated mitogenic indices of preeclamptic plasma were demonstrable months before the patients satisfied the clinical diagnosis of preeclampsia. Finally, differences in mitogenic index between the two patient groups disappeared by 6 weeks post partum, when clinical and biochemical evidence of the syndrome were no longer detectable. This is an especially important point because it excludes the possibility that the mitogenic index was increased in women with preeclampsia because of a temporary reduction in mitogenic activity in the immediate postpartum period. In addition, this finding suggests that altered mitogenic activity is a result of preeclampsia rather than a predisposing factor.

Several clinical and biochemical tests, including plasma fibronectin levels,[3] circulating factor VIII activity,[4] and response to angiotensin II infusion,[11] can identify women at risk for the development of preeclampsia by the midtrimester. Similar to the recent report on elevated levels of cellular fibronectin in preeclampsia,[12] the mitogenic index appears to statistically differentiate the group of patients in whom preeclampsia will develop as early as the first trimester of pregnancy. Because the most important pathophysiologic abnormalities of preeclampsia (poor trophoblast invasion and reduced placental perfusion[1]) are established early in pregnancy, it is not surprising that fundamental biochemical changes associated with this syndrome should also be detectable at this time.

Because the release of mitogenic growth factor activity is commonly associated with endothelial damage, these studies support our hypothesis that endothelial cell injury is an early, pathogenetic feature of preeclampsia.[1] Studies are currently underway to ascertain the identity and origin of the mitogenic activity. Partial purification of the mitogen(s) has indicated that this is a heat- and acid-labile protein with an apparent native molecular weight of 150,000.[13] This size is significantly larger than the classic growth factors and placental proteins with known trophic effects (e.g., human placental lactogen). However, the apparent molecular weight of this molecule(s) may reflect binding of a known growth factor to a plasma carrier protein, as in the case of insulin-like growth factors.[14] Future studies will attempt to ascertain how abnormal placentation, endothelial cell injury, and plasma mitogenic activity are linked in the pathophysiology of this puzzling syndrome.

We thank Mitra Jazayeri, Jean Perry, Alan Goldfien and the physicians of the Clinical Data Core for their support and expertise.

REFERENCES

1. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989;161:1200-4.
2. Spargo BH, McCartney CP, Winemiller R. Glomerular capillary endotheliosis in toxemia of pregnancy. Arch Pathol 1959;68:593-7.
3. Lazarchick J, Stubbs TM, Romein L, Van Dorsten JP, Loadholt CB. Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic. Am J Obstet Gynecol 1986;154:1050-2.
4. Redman CWG, Beilin LJ, Denson KWE, Bolton FG. Factor VIII consumption in preeclampsia. Lancet 1977; 2:1249-52.
5. Benedetto C, Bussolino F, Massobrio M, Camussi G. Prostacyclin and EPH gestosis. In: Salvatore B, ed. Fetal and postnatal outcome in EPH-gestosis. Amsterdam: Excerpta Medica, 1982:113-4.
6. Brown MA, Zammit VC, Lowe SA. Capillary permeability and extracellular fluid volumes in pregnancy-induced hypertension. Clin Sci 1989;77:599-604.
7. Rodgers GM, Taylor RN, Roberts JM. Preeclampsia is associated with a serum factor cytotoxic to human endothelial cells. Am J Obstet Gynecol 1988;159:908-14.
8. Musci TJ, Roberts JM, Rodgers GM, Taylor RN. Mitogenic activity is increased in the sera of preeclamptic

women before delivery. AM J OBSTET GYNECOL 1988; 159:1446-51.
9. DiCorleto PE. Cultured endothelial cells produce multiple growth factors for connective tissue cells. Exp Cell Res 1984;153:167-72.
10. Chesley L. Diagnostic criteria for preeclampsia. Obstet Gynecol 1985;65:423-5.
11. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest 1973;52: 2682-9.
12. Lockwood CJ, Peters JH. Increased plasma levels of ED1+ cellular fibronectin precede the clinical signs of preeclampsia. AM J OBSTET GYNECOL 1990;162:358-62.
13. Taylor RN, Musci TJ, Kuhn RW, Roberts JM. Partial characterization of a novel growth factor in the blood of women with preeclampsia. J Clin Endocrinol Metab 1990;70:1285-91.
14. Hintz RL, Liu F. Demonstration of specific plasma protein binding sites for somatomedin. J Clin Endocrinol Metab 1977;45:988-95.

The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term

Michael M. Moretti, MD, Fiona M. Fairlie, MD, Sherif Akl, MD, Aldo D. Khoury, MD, and Baha M. Sibai, MD

Memphis, Tennessee

Twenty patients with preeclampsia at a gestational age of 26 to 35 weeks were treated with oral nifedipine until delivery. The mean oral daily dose was 45.1 ± 11 mg/day (range, 40 to 80 mg/day). Fetal aorta, internal carotid artery, umbilical artery, and uteroplacental Doppler flow velocity waveforms were recorded before treatment and then serially. The mean nifedipine concentration at the time of the Doppler studies was 60.3 ng/ml (range, 10 to 90 ng/ml). The use of nifedipine therapy was associated with a significant decrease in both maternal systolic blood pressure (baseline, 154 to 135 mm Hg, $p < 0.001$) and diastolic blood pressure (baseline, 100 to 88 mm Hg, $p < 0.001$). However, there was no significant difference in the resistance index between baseline and postnifedipine Doppler studies in either the fetal or uteroplacental vessels. The use of oral nifedipine to control blood pressure in preeclampsia does not affect the resistance indices in fetal or uteroplacental vessels as measured by the Doppler technique. (AM J OBSTET GYNECOL 1990;163:1844-8.)

Key words: Preeclampsia, nifedipine, Doppler velocity waveforms

Nifedipine, a dihydropyridine calcium channel blocker, is a particularly effective antihypertensive agent.[1] The use of calcium channel blockers in the pregnant animal model was reportedly associated with a decrease in placental blood flow and development of fetal hypoxemia and acidosis.[2-6] However, their use to treat preterm labor and in the management of hypertensive disorders of pregnancy was not associated with any reported adverse perinatal outcome.[7-10]

Three reports described the acute effects of calcium channel blockers on fetal and uteroplacental circulations in hypertensive pregnancies. All three studies evaluated the effects of a single dose of either nifedipine ($n = 2$)[8, 10] or nicardipine ($n = 1$).[9] None of these studies reported any adverse effects on either fetal or uteroplacental circulations. In addition, Mari et al.[7] reported the effects of nifedipine therapy for preterm labor and concluded that short-term nifedipine therapy did not influence either fetal or uteroplacental circulation as evaluated with Doppler technique. To date, there are no reports describing the effects of long-term oral nifedipine therapy on fetal and uteroplacental circulations.

The purpose of this study was to examine the effects on fetal and uteroplacental Doppler velocimetry waveforms of long-term oral nifedipine therapy in patients with preeclampsia remote from term.

Material and methods

The study was performed at E.H. Crump Women's Hospital and Perinatal Center between 1988 and 1989.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Tennessee, Memphis.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Michael Moretti, MD, 853 Jefferson Ave., Room E102, Memphis, TN 38103.
6/6/24730

The study population included 20 patients with documented preeclampsia. All patients gave written consent for the study, which was approved by the Institutional Investigational Review Board. Entry criteria included the following: maternal age ≥15 years, gestational age between 26 and 35 weeks with a persistent maternal systolic blood pressure range of 140 to 170 mm Hg and a diastolic blood pressure range of 90 to 110 mm Hg 24 hours after hospitalization, the development of proteinuria ≥300 mg/24 hours, and documented normal first-trimester blood pressure readings. The exclusion criteria of the study included the presence of twins, diabetes mellitus, Rh isoimmunization, and congenital fetal malformation. In addition, any pregnancy complicated by signs of intrauterine growth retardation or fetal distress was excluded. Intrauterine growth retardation was defined as an estimated fetal weight by ultrasonographic measurement of head circumference and abdominal circumference below the 10th percentile for our population standards.

All patients were admitted to the antepartum unit and placed at bed rest. Blood pressure measurements were performed four times daily. No other medications were used except prenatal vitamins and iron supplementation.

Nifedipine therapy was started at 10 mg orally every 6 hours (40 mg/day) and increased to a maximum dosage of 80 mg/day. The dosage was titrated to keep the systolic blood pressure <140 mm Hg and diastolic blood pressure <90 mm Hg. Serum nifedipine levels were tested after at least 48 hours (eight doses) of dosing to achieve steady state and at the time of Doppler studies.

Baseline Doppler studies were performed immediately before treatment, repeated after steady state (48 hours), and then at 3-day intervals until delivery. Doppler studies were scheduled for 1 hour after the midday nifedipine dose. The Doppler ultrasonographic studies were done with an Ultramark 9 (ATL) system. A 50 Hz wall filter was used; Doppler ultrasonographic energy was 114 mw/cm^2 spatial peak temporal average intensity. The fetuses were examined with the mothers in semirecumbent position. The uterine arteries were studied with continuous-wave technique as described by Schulman et al.[11] Measurements from both uterine vessels were obtained and the results were averaged. Umbilical arteries were studied by pulsed Doppler with the sample volume placed in a free loop midportion of the cord. The fetal descending aorta was examined in the absence of fetal breathing movements at a point just above the level of the diaphragm with the transducer oriented to maintain the angle of insonation between 40 and 60 degrees. A sufficiently large sample volume was chosen to completely cross the aorta but not to provide interference with other vessels. Waveforms were recorded over a 5-second period that included an average of 10 consecutive cadiac cycles. Doppler velocity waveforms of the intracranial portion of the fetal internal carotid artery at the level of the bifurcation into the middle and anterior cerebral artery were recorded by the method described by Wladimiroff et al.[12] To determine these waveforms, we obtained the standard plane for measuring the biparietal diameter. The transducer was then moved parallel down to the base of the skull until a plane was reached that demonstrated the brain stem with the cerebral peduncles. Anterior to the heart-shaped brain stem on either side of the midline, a cross-section of the internal carotid artery as it divides into the anterior and middle cerebral vessels was seen pulsating. Color flow Doppler was used to help define these vessels. The sample volume (4 mm) was placed over the internal carotid artery and the maximum systolic velocity was recorded. Doppler waveforms were quantified by calculating the resistance index (Peak systolic velocity − Diastolic velocity/Peak systolic velocity).

Delivery data included placental weight, birth weight, Apgar scores, and cord arterial blood gases. Results were analyzed with analysis of variance for repeated measurements. A probability value of <0.05 was considered to indicate statistical significance.

Results

A total of 20 patients were studied; 16 were primigravid and four were multigravid women. The mean maternal age was 23 ± 6.23 years. Relevant clinical findings of the study patients are summarized in Table I. The mean nifedipine dose was 45.1 mg/day, and the mean interval between starting treatment and the first postdrug Doppler study was 95 hours. The mean maternal serum nifedipine concentration performed at the time of Doppler studies was 60.3 ng/ml (range, 10 to 90 ng/ml). Nifedipine therapy was associated with a significant decrease in maternal blood pressure. Mean systolic pressure decreased from 154 to 135 mm Hg ($p < 0.001$). Mean diastolic pressure decreased from 100 to 88 mm Hg ($p < 0.001$).

Results of the Doppler studies are summarized in Table II. All pretreatment and posttreatment Doppler results were within 1 SD of the mean for our normal population. There was no significant change in the resistance index between the baseline and Doppler studies performed at 4 days after nifedipine therapy. In addition, there were no differences in the resistance indices in subsequent measurements (by analysis of variance for repeated measurements).

Perinatal outcomes are listed in Table III. There were four (20%) small-for-gestational-age infants. Among these four pregnancies, maternal mean systolic pressure decreased from 156 to 131 mm Hg and mean

Table I. Clinical findings ($N = 20$)

	Mean ± SD	*Range*
Admission gestational age (wk)	31.0 ± 3.1	26-35
Delivery gestational age (wk)	33.9 ± 3.4	31-39
Pregnancy prolongation (days)	21.4 ± 20	5-84
Dose of nifedipine (mg/day)	45.1 ± 11	40-80

Table II. Doppler studies (resistance index)

	Baseline (mean ± SD)	*Treatment* (mean ± SD)*
Umbilical artery	0.61 ± 0.1	0.70 ± 0.8
Uterine artery	0.59 ± 0.1	0.57 ± 0.15
Descending aorta	0.85 ± 0.04	0.84 ± 0.05
Internal carotid artery	0.77 ± 0.08	0.69 ± 0.11

*At time of initial Doppler measurement performed an average of 4 days after therapy.

diastolic blood pressure decreased from 96 to 83 mm Hg in response to nifedipine therapy. These changes were not significantly different to the group as a whole. One infant had an acidotic cord arterial pH. This infant was delivered by cesarean section at 28 weeks' gestational age because of repetitive variable decelerations in labor.

Comment

Preeclampsia is a disease that is unique to human pregnancy, with an unknown cause. Despite recent progress in understanding its pathophysiology, the available therapeutic tools remain limited to either bed rest or delivery. Thus there remains an important place for the development of new approaches, such as calcium channel blockers, to the management of preeclampsia.

The results of a number of animal studies with calcium channel blockers are concerning. In addition, in both animal and human investigations there is a heterogeneity with regard to the specific calcium channel blockers studied. Although both nifedipine and nicardipine are dihydropyridine calcium channel blockers, they are biochemically different.[15] Caution should be exercised in extrapolating the effects of nicardipine to nifedipine. Parisi et al.[2,3] studied the effects of maternal nicardipine therapy on fetal placental blood flow and cardiorespiratory status in the hypertensive pregnant ewe. After maternal infusion of angiotensin II, nicardipine was infused and fetal and maternal blood flow were measured. Nicardipine reversed maternal hypertension but placental blood flow decreased and vascular resistance increased. They noted the death of five of 15 fetuses 65 minutes after nicardipine infusion. Ducsay et al.[4] administered nicardipine to spontaneously

Table III. Perinatal outcome

	Mean ± SD	*Range*
Placental weight (gm)	559 ± 154	260-965
Birth weight (gm)	2031 ± 743	613-3418
Apgar scores		
1 min	7.1 ± 2.0	1-9
No. with score ≤4	3	
5 min	8.7 ± .5	8-9
Cord arterial pH	7.28 ± .05	7.16-7.38
Cord arterial P_{CO_2}	51.3 ± 3.0	47-60
Cord arterial P_{O_2}	25.7 ± 9.8	13-40
Base excess	−2.7 ± 2.7	0.5−−7.1

contracting rhesus monkeys and noted the development of fetal acidosis and hypoxemia. Ahokas et al.[14] used a different calcium channel blocker, nifedipine, and found that blood flow was not significantly altered by nifedipine in the spontaneously hypertensive rat.

There have been three reports of studies of the effects of calcium channel blockers on the uteroplacental and umbilical circulations in hypertensive human pregnancies. Cameron et al.[9] studied the effects of nicardipine when used for the acute reduction of blood pressure in patients with preeclampsia. There were no significant alterations in either the pulsatility index or resistance index of the uteroplacental or umbilical vessels within 60 minutes of a single oral dose of nicardipine. Lindow et al.[8] determined an index of uteroplacental blood flow by use of a radioisotope method. There was no significant change in the blood flow index after a single dose of nifedipine despite a significant fall in blood pressure. In addition, Hanretty et al.[10] were unable to demonstrate a change in Doppler waveforms within 8 hours of a single 20 mg oral dose of nifedipine in nine pregnant women with proteinuric hypertension. Recently Mari et al.[7] performed Doppler studies of the uteroplacental and fetal circulations in patients with preterm labor who were treated with nifedipine for tocolysis. No significant difference was found in flow velocity waveforms 5 hours after the initial dose. The results of these studies suggest that short-term nifedipine therapy does not influence fetal or uteroplacental circulations.

This study was designed to investigate the effects of long-term oral nifedipine therapy on the uteroplacental and fetal circulations in hypertensive pregnancies by means of the Doppler technique. In our study long-term oral nifedipine therapy was associated with a significant decrease in maternal blood pressure. We were unable to demonstrate a significant difference in the resistance indices between baseline and first postnifedipine Doppler studies. In addition, when performed serially, there was no significant change in subsequent Doppler indices in any of the vessels studied. Although we were unable to demonstrate a change in resistance

as measured by the Doppler technique, it should be noted that there may still be a decrease in perfusion or flow to the vascular beds studied, based on a decrease in perfusion pressure to the uteroplacental circulation. This may not be manifested by a change in the Doppler resistance indices. Trudinger et al.[15] compared Doppler flow velocity waveforms with microsphere blood flow data in the umbilical placental circulation after the administration of the vasodilator prostacyclin. They observed a fall in the systolic/diastolic ratio of the fetal umbilical artery as measured by the Doppler technique. However, measurements of the umbilical placental blood flow by the radioactive microsphere technique showed a decrease in umbilical placental blood flow. Their results were similar to those of Parisi and Walsh,[15] who demonstrated a fall in fetal mean arterial pressure during prostacyclin infusion. Placental blood flow decreased, whereas vascular resistance was unchanged. Thus there could be a potential decrease in flow as a result of decreases in perfusion pressure. This could occur in the absence of alterations in resistance as measured by the Doppler technique. However, increases in the resistance index and systolic/diastolic ratio of uteroplacental circulation (as measured by the Doppler technique) have been associated with adverse pregnancy outcome. There were no significant changes in any of the vessels studied in response to treatment. This is further supported by the overall favorable perinatal outcome of the group.

It should be noted that all pretreatment Doppler study results were normal for gestational age. In addition, none of the pregnancies was complicated by signs of fetal growth failure or fetal compromise before the start of therapy, as determined by ultrasonographic measurements and biophysical testing. Thus the effect of long-term nifedipine therapy in hypertensive pregnancies with such complications cannot be established by these data. However, it must be noted that four (20%) of the infants were small for gestational age at delivery, and none demonstrated a significant change in the Doppler waveforms in any of the vessels studied. In addition, no fetus had signs of fetal distress in the antepartum period.

It has been demonstrated in the lamb model that the fetus responds to hypoxemia and acidemia with a redistribution of cardiac output from the splanchnic circulation that results in an increase in flow to the brain and heart. Low peripheral resistance in the nonhypoxic state is replaced by selective vasoconstriction mediated by α-adrenergic innervation to the gut, kidney, and lung.[17, 18] Our results show no significant change in the resistance index of the cerebral vessels or descending aorta. This lack of change suggests that there was no significant redistribution of cardiac output as a result of fetal hypoxemia. This is further supported by the perinatal outcome in our study group; only one infant had an acidotic cord arterial pH at delivery.

Plasma levels of nifedipine were performed at the time of Doppler studies. The first Doppler studies were performed 48 hours after the start of therapy to measure flow velocity waveforms in the steady state. Doppler studies were scheduled 1 hour after the midday dose. Maximum activity concentration in the serum are reached 1 to 2 hours after administration of nifedipine.[19] Thus, although we regret that we cannot report the Doppler findings at initial peak serum levels, efforts were made to perform the Doppler studies at a time of maximum activity concentration. Maternal plasma nifedipine levels ranged from 10 to 90 ng/ml. Therapeutic effects of nifedipine have been reported with plasma values as low as 3 ng/ml.[19] This demonstrates that nifedipine did not influence Doppler waveforms when present in the maternal circulation in therapeutic concentrations. This is further supported by the significant decrease in maternal blood pressure during therapy.

We conclude that long-term oral nifedipine therapy in patients with preeclampsia effectively controls maternal hypertension with no effect on fetal or uteroplacental Doppler indices. Whether the same results can be obtained during the chronic use of nifedipine in pregnancies complicated by fetal growth failure or compromise remains to be established.

REFERENCES

1. Walters BNJ, Redman CWG. Treatment of severe pregnancy associated hypertension with the calcium antagonist nifedipine. Br J Obstet Gynaecol 1984;91:330-6.
2. Parisi VM, Salinas JM, Stockmar EJ. Placental vascular responses to nicardipine in the hypertensive ewe. Am J Obstet Gynecol 1989;161:1039-43.
3. Parisi VM, Salinas JM, Stockmar EJ. Fetal vascular responses to maternal nicardipine administration in the hypertensive ewe. Am J Obstet Gynecol 1989;161:1035-8.
4. Ducsay CA, Thompson JS, Wu AT, Novy MJ. Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes. Am J Obstet Gynecol 1987;157:1482-6.
5. Tsujiei M, Furuhashi N, Takahashi T, Yajima A. The effect of nifedipine on placental perfusion in the Wistar rat. In: Abstracts of the sixth international congress of the International Society for the Study of Hypertension in Pregnancy. Montreal, Quebec, Canada, 1988:138.
6. Laupe LG, Princzkel E, Vojek L. Effects of nifedipine on the foetal cardiorespiratory system in pregnant sheep. In: Abstracts of the sixth international congress of the International Society for the Study of Hypertension in Pregnancy. Montreal, Quebec, Canada, 1988:140.
7. Mari G, Kirshon B, Moise K, Lee W, Cottom DB. Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor. Am J Obstet Gynecol 1989;161:1514-8.
8. Lindow SW, Davies N, Davey DA, Smith SA. The effect of sublingual nifedipine on uteroplacental blood flow in hypertensive pregnancy. Br J Obstet Gynaecol 1988;95:1276-81.
9. Cameron AD, Walker JJ, Mathara AM, Bjornsson S. The

effect of antihypertensive therapy on the doppler waveform in the maternal and fetal vascular system. In: Abstracts of the second International Doppler Society meeting, Paris, France, 1989:7.
10. Hanretty KP, Whittle JM, Howie CA, Rubin PC. Effect of nifedipine on doppler flow velocity waveforms in severe preeclampsia. Br Med J 1989;299:1205-6.
11. Schulman H, Fleischer A, Farmakides G, et al. Development of uterine artery compliance in pregnancy as detected by Doppler ultrasound. AM J OBSTET GYNECOL 1985;155:1031-6.
12. Wladimiroff JW, Tonge HM, Stewart PA. Doppler assessment of cerebral blood flow in the human fetus. Br J Obstet Gynaecol 1986;93:851.
13. Goodman LS, Gilman AG, Rall TW, Murad F, eds. The pharmacological basis of therapeutics. 7th ed. New York: MacMillan, 1985.
14. Ahokas RA, Sibai BM, Mabie WC, Anderson GD. Nifedipine does not adversely effect uteroplacental blood flow in the hypertensive term-pregnant rat. AM J OBSTET GYNECOL 1988;159:1440-5.
15. Trudinger BJ, Connelly AJ, Giles WB, Hales JR, Wilcox GR. The effects of prostacyclin and thromboxane analogue (U46619) on the fetal circulation and umbilical flow velocity waveforms. J Dev Physiol 1989;11(3):179-84.
16. Parisi VM, Walsh SW. Fetal vascular responses to prostacyclin. AM J OBSTET GYNECOL 1989;160:871-8.
17. Cohn HG, Sacks EJ, Heyman MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. AM J OBSTET GYNECOL 1974;120:817-24.
18. Richardson BS, Carmichael L, Jacobus H, Taraswell K, Webster A. Regional blood flow change in the lamb during the perinatal period. AM J OBSTET GYNECOL 1989; 160:919-25.
19. Banzet O, Colin JN, Thibonnier M, Singlas E, Alexandre JM, Corvol P. Acute antihypertensive effect and pharmacokinetics of a tablet preparation of nifedipine. Eur J Clin Pharmacol 1983;24:145-50.

Neuroophthalmologic effects of intravenous magnesium sulfate

Kathleen B. Digre, MD,[a] Michael W. Varner, MD,[b] and Jade S. Schiffman, MD, LTC, MC, USA[c]

Salt Lake City, Utah, and San Francisco, California

To test the hypothesis that visual disturbances are more common during intravenous magnesium sulfate administration than at 1 to 4 days after discontinuation of the drug, 13 women underwent beoside neuroophthalmologic examinations during intravenous magnesium sulfate tocolysis at 2.0 to 3.0 gm/hr and again at 1 to 4 days after cessation of therapy. Visual symptoms were common during intravenous magnesium sulfate administration. Blurred vision was present in 12 of 13 patients and diplopia was present in 10 of 13 patients. Abnormal findings during neuroophthalmologic examination occurred in all patients during intravenous magnesium sulfate administration. Findings included ptosis, accommodative and convergence insufficiency, and abnormal pupillary responsiveness to light and near. All patients were symptom-free and had normal examinations after magnesium sulfate was discontinued. These findings suggest that visual disturbances with therapeutic magnesium sulfate are common. (AM J OBSTET GYNECOL 1990;163:1848-52.)

Key words: Magnesium sulfate, neuroophthalmology, visual disturbances

Intravenous magnesium sulfate is routinely administered in the United States to pregnant women with preeclampsia and eclampsia for seizure prophylaxis. Visual disturbances are sometimes seen in patients with preeclampsia and are considered a distinguishing criterion for severe disease.[1] In our population the most common visual symptoms associated with severe preeclampsia include visual blurring and visual field defects.[2] Visual disturbances in preeclampsia may be due to choroidal ischemia with serous retinal detachment (with or without evidence of other manifestations of hypertensive retinopathy) or cerebral vasospasm. However, the neuroophthalmologic effects of intravenous magnesium sulfate also could be responsible for visual symptoms. This possibility was suggested to us by the high frequency with which we have encountered visual complaints in pregnant women on our service receiving intravenous magnesium sulfate tocolysis. Recent studies have demonstrated that intravenous magnesium sulfate is an efficacious and relatively safe tocolytic agent with visual complaints reported infrequently.[3-6] Most patients with severe preeclampsia receive magnesium sulfate. As we were not certain which ophthalmologic

From the Departments of Neurology,[a] Ophthalmology,[a] and Obstetrics and Gynecology,[b] University of Utah School of Medicine, and the Department of Ophthalmology, Letterman Army Medical Center.[c]
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Michael W. Varner, MD, Department of Obstetrics and Gynecology, University of Utah School of Medicine, 50 North Medical Dr., Salt Lake City, UT 84132.
6/6/24725

symptoms and signs were due to magnesium sulfate alone, we decided to test the hypothesis that patients receiving intravenous magnesium sulfate tocolysis have more frequent visual signs and symptoms than at 1 to 4 days after discontinuation of the drug. The study design was approved by the Human Subjects Study Institutional Review Board of the University of Utah School of Medicine. Informed written consent was obtained from all patients before their entry to this study.

Material and methods

Patients admitted in premature labor to the Obstetric Service of the University of Utah Hospital between July 1, 1987, and April 15, 1989, were evaluated. On our service tocolysis is generally recommended for women presenting in labor before 35 weeks' gestation who have no maternal contraindications to continuing pregnancy and who have an appropriately grown, non-anomalous, immature, healthy fetus. Although β-sympathomimetic medications represent the usual initial tocolytic agent on our service, intravenous magnesium sulfate is occasionally used as the initial drug and may be given as an alternative tocolytic drug if a patient does not respond to β-sympathomimetics or experiences unacceptable side effects.

Our protocol for intravenous magnesium sulfate tocolysis is identical to that described by Dudley et al.[6] and consists of a 4.0 gm loading dose administered over 15 to 20 minutes, followed by a continuous intravenous infusion pump administration of 2.0 gm/hr. If patients are clinically stable but require extended magnesium sulfate tocolysis, they may be transferred to the antepartum ward if the dosage is ≤2.5 gm/hr. Higher levels require continued observation in the labor and delivery area. All patients receiving magnesium sulfate tocolysis on our service are assessed at least every 4 hours for routine vital signs, presence and nature of deep tendon reflexes, and subjective complaints of weakness, nausea, fatigue, headache, visual disturbances, difficulty breathing, chest pain, confusion, and difficulty with concentration.

Patients who were clinically stable yet required continued intravenous magnesium sulfate tocolysis were approached for entry into the study. Criteria for exclusion included coexistent administration of other tocolytic drugs, evidence of regular uterine activity within the preceding 12 hours, a history or evidence of underlying renal disease, coexistent preeclampsia, or the requirement of >2.5 gm/hr of intravenous magnesium sulfate tocolysis. Additionally, patients with a preconceptional history of decreased vision or stabismus were excluded from the study. Thirteen women qualified and consented to our study. They ranged from 13 to 35 years old (mean, 27.8 years). These patients were carefully interviewed during and after magnesium sulfate tocolysis. Specific questioning was done in regard to blurring at distance or near (monocular impairment of vision) and double vision (diplopia or binocular impairment of vision) at distance, near, or any particular gaze direction. Patients were also asked about scotomas or phosphenes.

A bedside neuroophthalmologic examination was performed on each woman during intravenous magnesium sulfate administration (study examination). The entire examination was repeated 1 to 4 days after the medication was stopped (control examination). In this fashion each woman served as her own control. Specific details of the neuroophthalmologic examination are available from the authors on request.

Statistical analysis was performed with the χ^2 analysis for discrete variables and Student's t test, with the Yates correction, for continuous variables. Statistical significance was defined by a p value <0.05.

Results

Serum magnesium levels at the time of study examination averaged 6.1 mg/dl, ranging from 4.7 to 7.4 mg/dl. As listed in Table I, visual symptoms were common in women receiving intravenous magnesium sulfate tocolysis. Other non-visual-tract symptoms were also common at the study examination, although all symptoms had resolved by the time of control reexamination.

All patients had clinically normal deep tendon reflexes, respiratory rates, and heart rates at study and control examinations.

With the exceptions of the Amsler grid, visual fields, versions, and pursuits, visual signs were all significantly affected during intravenous magnesium sulfate tocolysis (Table I). All abnormalities returned to values consistent with those of normal, healthy young women at the time of control reexamination.

Comment

Our protocol for magnesium sulfate tocolysis is identical to that of previous reports and our average serum levels correlate well with the data of others.[3,4] These levels are also within the generally accepted therapeutic range for seizure prophylaxis in women with preeclampsia.[1]

The adverse effects of hypermagnesemia that are commonly discussed include decreased deep tendon reflexes, muscle weakness, respiratory depression, and cardiac arrhythmias. With the exception of muscular weakness, none of these adverse effects were encountered, consistent with the previous observation that magnesium sulfate is associated with a low frequency of serious complications when carefully administered to appropriate candidates.[7]

In spite of our apparently similar drug administra-

Table I. Symptoms and signs in patients receiving intravenous magnesium sulfate tocolysis

	Study examination (N = 13)	*Control examination (N = 13)*	*p Value*
Visual symptoms			
Blurred vision	12	0	<0.0001
Diplopia	10	0	<0.0001
Scotomas-phosphenes	0	0	—
Photophobia	4	0	0.03
Other symptoms			
Headache	11	0	<0.0001
Nausea	5	0	0.015
Generalized weakness	3	0	0.06
Impaired concentration-confusion	3	0	0.06
Visual signs			
Abnormal visual acuity	5	0	0.015
Abnormal visual fields	0	0	—
Abnormal Amsler grid	0	0	—
Heterophorias	10	1	0.005
Abnormal versions	0	0	—
Abnormal pursuit	0	0	—
Hypometric saccade	10	0	<0.0001
End-gaze nystagmus	10	1	0.005
Pupillary responsiveness (mm)			
To light	0.5 (Range −0.1-1.0)	1.1 (Range 0.8-1.7)	0.002
To best near effort	0.4 (Range 0.1-1.0)	1.4 (Range 0.5-3.1)	0.0001
Remote NPA (cm)			
OD	14.2 (Range 9-19)	6.6 (Range 4-11)	<0.0001
OS	14.7 (Range 9-24)	6.8 (Range 4-11)	<0.0001
Marginal reflex distance	3.8 (Range 2.5-5.3)	5.3 (Range 4.0-6.5)	0.02
Fusional convergence amplitudes (diopter)			
Distance	6.7 (Range 4-14)	12.2 (Range 4-18)	0.005
Near	8.3 (Range 2-18)	23.0 (Range 8-40)	0.002
Fusional divergence amplitudes (diopter)			
Distance	6.1 (Range 4-9)	8.2 (Range 4-18)	0.01
Near	7.7 (Range 2-12)	15.4 (Range 6-25)	0.004

NPA, Near point of accommodation; *OD*, right eye; *OS*, left eye.

tion protocol, we have encountered a much higher incidence of visual signs and symptoms than have other investigators. Hollander et al.[3] reported a finding of diplopia in only two of 33 patients and no other adverse visual effects, and other recent evaluations[7,8] did not report any adverse visual signs or symptoms.

Our patients experienced frequent visual complaints (blurred vision, double vision) during a magnesium sulfate tocolysis protocol where the dose was similar to that of the aforementioned authors. It is unclear why we should have such different findings, although the possibility certainly exists that specific questioning may have increased the incidence of positive responses.

We found a consistent pattern of neuroophthalmologic abnormalities during intravenous magnesium sulfate administration. The most striking finding was bilateral symmetric ptosis. Levator function was normal, suggesting a supranuclear mechanism.

Fusional convergence amplitudes were less than half of control measurements. A less dramatic, but significant finding was the decrease in fusional divergence amplitudes during magnesium sulfate administration. The fact that both convergence and divergence amplitudes were diminished in the setting of grossly normal versions also speaks for a supranuclear disturbance in the control of motor fusion.

We found that the majority of our patients had an exodeviation at near. This, combined with poor fusional convergence amplitudes, defined convergence insufficiency in our patients. This is also a presumably supranuclear disturbance. Additionally, all patients had a remote near point of accommodation and therefore had an accommodative insufficiency. The accommodative insufficiency could result from either a supranuclear or a peripheral site of dysfunction.

We hypothesize that intravenous magnesium sulfate causes neuroophthalmologic dysfunction and that both central and peripheral mechanisms may be involved. The abnormalities of convergence, accommodation, and pupillary testing also suggest both central and peripheral mechanisms. Further studies will be required to determine what proportion of these changes is mediated by peripheral effects of magnesium sulfate on the myoneural junction and what proportion might be

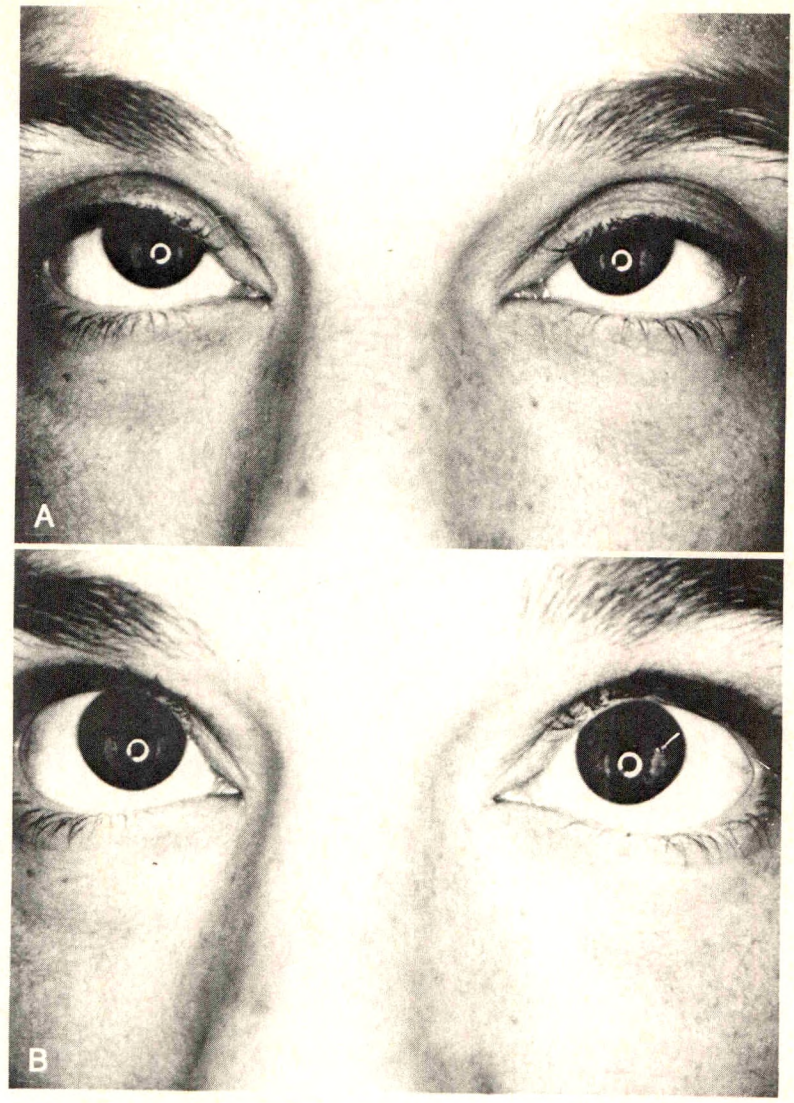


Fig. 1. Standardized Polaroid photographs of the same woman **(A)** while receiving intravenous magnesium sulfate tocolysis and **(B)** at control reexamination (after intravenous magnesium sulfate tocolysis).

mediated via central effects. Such studies might also provide valuable insights into the potential anticonvulsant effects of magnesium sulfate.

We have presented the ophthalmologic symptoms and signs of intravenous magnesium sulfate administration. It is well known that visual symptoms can develop in women with severe preeclampsia and eclampsia and that this may indicate worsening of the condition. As these women are treated with magnesium sulfate, it is important to differentiate the visual complaints from worsening of disease from those of intravenous magnesium sulfate therapy. We have previously found that women with severe preeclampsia and/or eclampsia complain primarily of scotomas (both positive and negative) and/or segmental visual blurring,[2] unlike the complaints of blurred vision and diplopia found in this study. These symptoms were frequently due to associated serous retinal detachments and choroidal infarctions that were not present in the patients in this study. The Amsler grid has been found to be of value in identifying women with preeclampsia who have scotomas and has not demonstrated any abnormalities in women undergoing magnesium sulfate tocolysis.

We have demonstrated visual symptoms and signs associated with intravenous magnesium sulfate administration. Magnesium sulfate produces blurred vision and/or diplopia but is not associated with scotoma or phosphenes. Although blurred vision may be seen in preeclampsia, abnormalities or Amsler grid testing are

more common. This is important to understand, since the obstetrician can usually differentiate between the two by history and simple bedside examination techniques, to make appropriate management decisions. While obstetricians may not be conversationally familiar with all of the definitions and procedures presented in this manuscript, these definitions and procedures represent standard ophthalmologic practice. It is important that obstetricians be aware of the visual abnormalities produced by magnesium sulfate because these abnormalities are common and obvious to careful observation.

REFERENCES

1. Cunningham FG, MacDonald PC, Gant NF. Williams' obstetrics. 18th ed. Norwalk, Connecticut: Appleton & Lange, 1989:674.
2. Digre KB, Varner MW, Servais EG, Durcan FJ. Neuro-ophthalmologic abnormalities in severe pre-eclampsia and eclampsia. Invest Ophthalmol Vis Sci 1989;30(suppl):245.
3. Hollander DI, Nagey DA, Pupkin MJ. Magnesium sulfate and ritodrine hydrochloride: a randomized comparison. Am J Obstet Gynecol 1987;156:631-7.
4. Elliott J. Magnesium sulfate as a tocolytic agent. Am J Obstet Gynecol 1983;147:277-84.
5. Petrie R. Tocolysis using magnesium sulfate. Semin Perinatol 1981;5:266-73.
6. Dudley D, Gagnon D, Varner MW. Long-term intravenous magnesium tocolysis. Obstet Gynecol 1989;73:373-8.
7. Wilkins IA, Lynch L, Mehalek KE, Berkowitz GS, Berkowitz RL. Efficacy and side effects of magnesium sulfate and ritodrine as tocolytic agents. Am J Obstet Gynecol 1988;159:685-9.
8. Spisso KR, Harbert GM, Thiagarajah S. The use of magnesium sulfate as the primary tocolytic agent to prevent premature delivery. Am J Obstet Gynecol 1982;142:840-5.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.

Low-dose aspirin

II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome

Charles E. L. Brown, MD, Norman F. Gant, MD, Kay Cox, MT(ASCP), Bernard Spitz, MD, Charles R. Rosenfeld, MD, and Ronald R. Magness, PhD

Dallas, Texas

Forty pregnant women (28 to 32 weeks' gestation) were given low-dose aspirin therapy (81 mg/day) from the time of enrollment until delivery; circulating eicosanoid levels and angiotensin II pressor responses were measured before and after 1 week of aspirin therapy. Subsequent clinical outcome was correlated with these results. All women had significant reductions in serum and plasma thromboxane B_2 levels with aspirin treatment ($p < 0.01$). Eleven women who remained sensitive to the pressor effects of angiotensin II (effective pressor dose < 10 ng/kg/min) after 1 week of low-dose aspirin treatment exhibited significant decreases ($p < 0.05$) in plasma 6-keto-prostaglandin $F_{1\alpha}$ (264 ± 119 vs 161 ± 31 pg/ml, mean ± SD) and prostaglandin E_2 (476 ± 174 vs 351 ± 112 pg/ml) levels. In contrast, patients who were either nonsensitive (refractory) to angiotensin II ($n = 18$; ≥10 ng/kg/min) before aspirin or became nonsensitive after aspirin administration ($n = 11$) had no change in either plasma 6-keto-prostaglandin $F_{1\alpha}$ or prostaglandin E_2 concentrations. The occurrence of pregnancy-induced hypertension was 100% in the women who remained angiotensin II sensitive during aspirin therapy as compared with 36% and 39% in the other two groups ($\chi^2 = 16.14$; $p < 0.001$). Thus during low-dose aspirin therapy a failure to develop refractoriness to infused angiotensin II is associated with a nonselective inhibition of eicosanoids and the almost certain development of pregnancy-induced hypertension. These observations may reflect a basic defect in vascular adaptation to pregnancy. (Am J Obstet Gynecol 1990;163:1853-61.)

Key words: Low-dose apsirin, preeclampsia, angiotensin II, pressor responses, prostaglandins, blood pressure control

Normal pregnancy is associated with a decrease in the systemic pressor response to infused angiotensin II.[1-3] In contrast, women either with or destined to have pregnancy-induced hypertension are sensitive to this vasoconstrictor.[2, 3] Prostaglandins[4-6] and, more specifically, a balance between prostacyclin and thromboxane A_2 may be contributing factors in the control of vascular refractoriness to the pressor effects of infused angiotensin II.[7-9] Since thromboxane A_2 has potent vasoconstrictive and platelet aggregating actions and is released by circulating platelets,[10] platelet- and/or cyclooxygenase-inhibiting drugs have been used in recent clinical trials to prevent preeclampsia. Such therapy has been reported to be clinically beneficial.[11-13] For example, in a randomized placebo-controlled study Wallenburg et al.[12] used low-dose aspirin therapy in a group of angiotensin II–sensitive primigravid women and observed a decrease in the occurrence of proteinuric hypertension (preeclampsia). However, treatment failures have been reported in each of these studies.[11-13] Recently, Spitz et al.[14] reported that low-dose aspirin therapy (81 mg/day) dramatically increased the prostacyclin/thromboxane A_2 ratio in the plasma of angiotensin II pressor–sensitive women and that this was associated with partial restoration of angiotensin II pressor refractoriness in some (53%) but not all of these women; that is, we observed a heterogeneous angiotensin II pressor response after 1 week of low-dose aspirin therapy. Because of the small numbers of patients stratified into the various angiotensin II–sensitive and nonsensitive subpopulations after low-dose aspirin therapy, we did not report data regarding clinical outcome.[14]

Although we[14] and others[15, 16] have demonstrated that low-dose aspirin therapy results in decreased sensitivity to infused angiotensin II in most pregnant women, these infusions are time-consuming, are invasive, and have the theoretic risk of causing a hypertensive emergency in women who might be exquisitely pressor sensitive. Thus large clinical trials involving low-dose as-

From the Departments of Obstetrics and Gynecology and Pediatrics, The University of Texas Southwestern Medical Center.
Supported by National Institutes of Health grants HD24971 and HD08783 and by American Heart Association Texas Affiliate grant 86R-078.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Ronald R. Magness, PhD, Department of Pediatrics and Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235.
6/6/24780

pirin therapy are being conducted currently in unselected populations. In the present study we administered low-dose aspirin throughout the third trimester of pregnancy and after 1 week we evaluated the effect of such therapy on circulating eicosanoids and angiotensin II pressor responsiveness in both angiotensin II pressor–sensitive and nonsensitive women; we also correlated these responses with subsequent clincial outcome. The hypothesis tested was that women who were sensitive to infused angiotensin II and remained sensitive to this pressor agent during low-dose aspirin therapy would have significant decreases in prostacyclin and pregnancy-induced hypertension would develop subsequently.

Material and methods

In this study aspirin (81 mg) was administered daily throughout the third trimester of pregnancy to women who were not sensitive to the pressure effects of angiotensin II (normal; $n = 18$) and women who were sensitive (future hypertensive; $n = 22$). Sensitivity to angiotensin II was defined as a pressor dose < 10 ng/kg/min; refractoriness was defined as a pressor dose ≥ 10 ng/kg/min.[3, 14] After 1 week of therapy, we evaluated changes in (1) the minimum amount of angiotensin II per kilogram per minute required to increase diastolic blood pressure 20 mm Hg (the effective pressor dose of angiotensin II), (2) the concentrations of thromboxane A_2 measured as the stable metabolite thromboxane B_2 in serum and plasma, (3) the plasma prostacyclin measured as the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$, and (4) plasma prostaglandin E_2. Angiotensin II pressor responses were measured in 18 nonsensitive women who served as controls; this was done to assess the effects of low-dose aspirin therapy on angiotensin II pressor responses, as well as circulating eicosanoids in normal pregnancies. The patients recruited for this study were nulliparous women from the antepartum clinics of Parkland Memorial Hospital, who were enrolled after exhibiting a positive rollover test. Some subjects also were recruited from women hospitalized with early-onset pregnancy-induced hypertension. In these women blood pressures had returned to normal ranges after being placed at bed rest. Candidates were excluded if they had a history of ingestion of any drugs other than iron supplementation or of chronic hypertension, heart disease, or diabetes. Prostaglandin data from 12 of the angiotensin II–sensitive women have been reported previously[14]; however, an additional aliquot of plasma and serum obtained from these women was assayed with the specimens obtained from the angiotensin II refractory women to decrease interassay variability. The women were included in the present study if they met these criteria: nulliparity, documented aspirin ingestion for the remainder of the pregnancy, and additional aliquots of blood that were available for prostaglandin assays. This study was approved by the institutional review board for human experimentation at The University of Texas Southwestern Medical Center.

The angiotensin II sensitivity test was performed as described previously.[3] Subjects were placed in the left lateral recumbent position in a quiet room. Blood pressure and heart rate were automatically measured from the right arm every 5 minutes with an automatic blood pressure cuff (Dinamap 1846 SX/P, Critikon, Inc., Johnson & Johnson, Tampa). After stabilization of the diastolic blood pressure for 15 minutes, an intravenous infusion was started in the left arm. Blood specimens for prostaglandin assays were collected as described below. After blood collection, the tubing was connected to an infusion pump with a syringe containing angiotensin II amide (1000 ng/ml in 0.9% sodium chloride, Ciba-Geigy Pharmaceuticals). The initial dose was 1 to 2 ng/kg/min; this was gradually increased at 5-minute intervals. The test was stopped after the diastolic blood pressure increased 20 mm Hg or if the dosage rate exceeded 40 ng/kg/min. After completion of the dose-response test and after blood pressures had returned to baseline values, the effective pressor dose of angiotensin II was infused again to confirm the pressure effect with that dose. The results in all studies were identical to the first pressor dose response.

After blood collection and the determination of angiotensin II sensitivity, the women were given a bottle of seven "baby" aspirin (81 mg) and instructed to take one aspirin daily for 1 week, at which time they were to return for a repeat examination. On return, pills were counted to confirm use. Those women in the hospital received aspirin as a daily medication. The procedures described above for blood collection and sensitivity to angiotensin II were repeated after 1 week of aspirin therapy. The women remained on daily low-dose apsirin therapy until the end of pregnancy. The remainder of pregnancy and labor and delivery were managed by obstetric house staff under the supervision of faculty not involved with this study. Clinical data regarding the outcome of the pregnancy, gestational age at delivery, Apgar scores, and birth weights were recorded. Pregnancy-induced hypertension was defined as the measurement of two diastolic blood pressures ≥ 90 mm Hg recorded at least 6 hours apart. Preeclampsia was defined as pregnancy-induced hypertension with the presence of proteinuria (at least 1+) on a voided urine specimen. Severe preeclampsia was defined as a diastolic blood pressure ≥ 110 mm Hg, and/or proteinuria (2+), and/or laboaratory evidence of end-organ damage.[17]

Prostaglandin assays. Serum concentrations of thromboxane B_2, which were reflective of platelet-derived thromboxane A_2, were obtained from blood samples after clotting (45 minutes at room tempera-

Table I. Initial clinical characteristics of nulliparous women undergoing angiotensin II infusion

	Nonsensitive	*Sensitive-nonsensitive*	*Sensitive-sensitive*
Age (yr)	18.8 ± 1.5 (n = 18)	19.5 ± 3.7 (n = 11)	18.6 ± 3.2 (n = 11)
Gestational age at 1st angiotensin II infusion (wk)	30.3 ± 2.8 (n = 18)	31.3 ± 2.6 (n = 11)	32.5 ± 1.6 (n = 11)
Weight (kg)	68.0 ± 12.7 (n = 18)[a]	65.3 ± 11.8 (n = 11)[a]	80.3 ± 13.6 (n = 11)[b]
Blood pressure (mm Hg)			
Systolic	98.1 ± 6.9 (n = 18)[a]	106.5 ± 11.1 (n = 11)[b]	112.2 ± 9.5 (n = 11)[b]
Diastolic	49.7 ± 5.5 (n = 18)[a]	57.3 ± 9.8 (n = 11)[b]	59.4 ± 8.2 (n = 11)[b]
Heart rate (beats/min)	87.1 ± 5.8 (n = 16)[a]	80.4 ± 11.6 (n = 8)[a, b]	75.1 ± 7.0 (n = 11)[b]

Patients are grouped according to pressor responses to angiotensin II. Values are means ± SD. Numbers in parentheses are numbers of observations. Means with different superscripts (a,b) are different ($p < 0.05$).

ture) and were used as an indication of therapy compliance. All patients had a fall in serum thromboxane B_2 levels of >60%. The assay for serum thromboxane B_2 has been described previously.[14] All samples were assayed in duplicate.

Blood samples (6.0 ml) for plasma prostanoids were obtained before initiation of the intravenous angiotensin II infusion, drawn into chilled syringes, transferred immediately to chilled tubes containing ethylenediaminetetraacetic acid (4.2 mg) and aspirin (0.3 mg), centrifuged at 5° C, and stored at −20° C until extraction, column chromatography, and radioimmunoassay. The method for measurement of plasma prostaglandins has been described and validated previously in our laboratory.[14]

Statistical analysis. All data are expressed as the mean ± 1 SD. Comparisons between groups were performed by the Student t test for paired and unpaired data or one-way analysis of variance, as appropriate. Nonparametric comparison between groups was performed by χ^2 or Fisher's exact test.

Results

Forty nulliparous women were enrolled and underwent angiotensin II infusions. Eighteen women were classified initially as nonsensitive (refractory) to angiotensin II whereas 22 women were sensitive to the angiotensin II infusion at the start of the study. These 22 women were further subdivided on the basis of the subsequent angiotensin II pressor response evaluated after 1 week of low-dose aspirin therapy. After 1 week of low-dose aspirin therapy, if the angiotensin II effective pressor dose equalled or exceeded 10 ng/kg/min, i.e., they became nonsensitive, they were grouped as angiotensin II–sensitive women responding favorably to aspirin therapy (sensitive-nonsensitive). There were 11 women in this group, five of whom had eicosanoid data reported previously.[14] After 1 week of low-dose aspirin therapy, if the angiotensin II sensitivity remained <10 ng/kg/min, they were classified as angiotensin II–sensitive women who were unresponsive to aspirin therapy (sensitive-sensitive). There were 11 women in this group, seven of whom had prostaglandin data reported previously.[14]

Listed in Table I are the clinical data for the three groups of pregnant women at the start of this study. All women were nulliparous and were of similar age and gestation at the start of the study. The angiotensin II–sensitive women who remained sensitive to the pressor effects of angiotensin II after 1 week of low-dose aspirin weighed 12 to 15 kg more than either of the other two groups ($p < 0.05$). Furthermore, the nonsensitive patients enrolled into this study had lower blood pressures and higher heart rates than the sensitive patients ($p < 0.05$). Women who became nonsensitive after 1 week of low-dose aspirin had an intermediate heart rate.

Platelet-derived serum thromboxane B_2 levels, an indicator of therapy compliance, fell markedly and similarly in all subjects in each group after 1 week of low-dose apsirin therapy [nonsensitive, 1084 ± 541 to 89 ± 63 pg/ml (92%); sensitive-nonsensitive, 1154 ± 746 to 79 ± 64 pg/ml (93%); sensitive-sensitive, 1655 ± 1780 to 196 ± 229 (88%) pg/ml; $p < 0.01$]. Plasma thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$, the stable metabolites of thromboxane A_2 and prostacyclin, respectively, as well as plasma prostaglandin E_2, also were measured. Before the start of aspirin therapy, the concentrations of each of these eicosanoids were similar in the three groups of patients subsequently stratified according to angiotensin II pressor sensitivity. The changes in these eicosanoids after 1 week of low-dose aspirin therapy are illustrated in Figs. 1, 2, and 3, respectively. Plasma thromboxane B_2 declined 61%, 60%, and 67% in the three study groups, respectively (Fig. 1). These changes in plasma and serum thromboxane A_2 levels after low-dose aspirin therapy were not significantly different among any of the three groups. Plasma 6-keto-prostaglandin $F_{1\alpha}$ levels were unchanged after 1 week of low-dose apsirin treatment in the non-

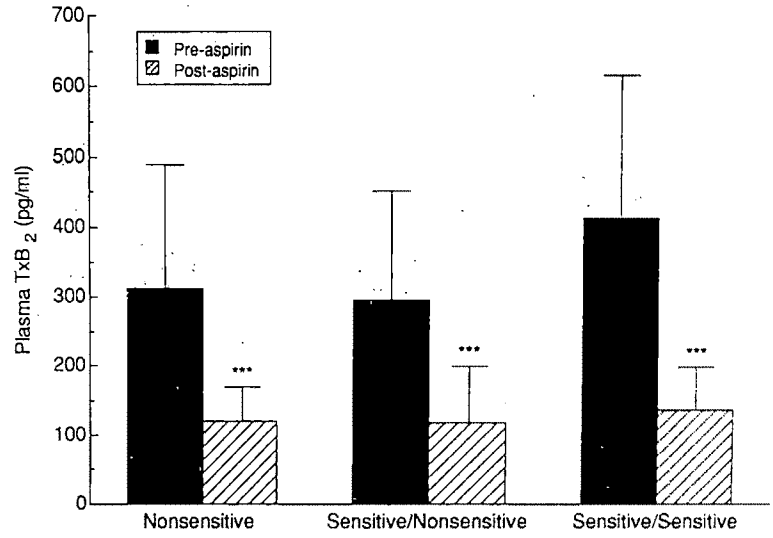


Fig. 1. Plasma concentrations of thromboxane B_2 in women who were sensitive or not sensitive to angiotensin II before and after low-dose aspirin therapy. *Triple asterisks,* $p < 0.001$ versus before aspirin.

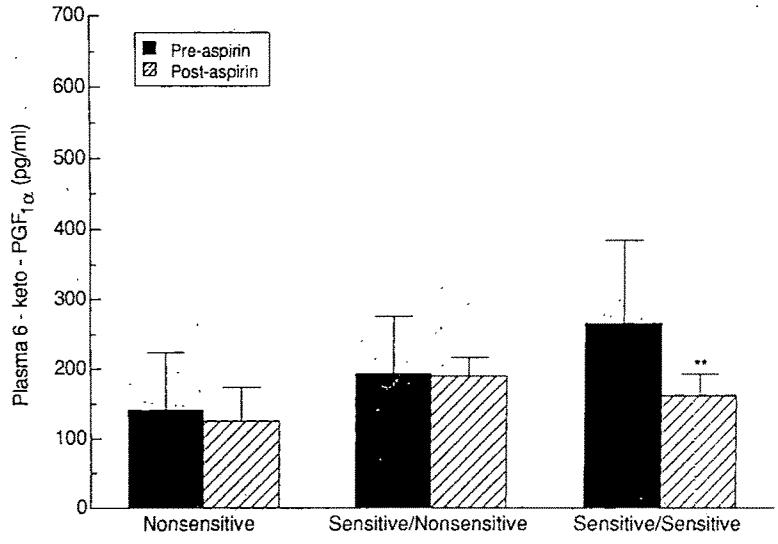


Fig. 2. Plasma concentrations of 6-keto-prostaglandin $F_{1\alpha}$ in women who were sensitive or not sensitive to angiotensin II before and after low-dose aspirin therapy. *Double asterisks,* $p = 0.016$ versus before asprin.

sensitive (142 ± 82 and 126 ± 47 pg/ml) and sensitive-nonsensitive (193 ± 82 and 189 ± 27 pg/ml) women; however, in the sensitive-sensitive group, plasma 6-keto-prostaglandin $F_{1\alpha}$ levels decreased 39%, from 264 ± 119 to 161 ± 31 pg/ml ($p < 0.05$; Fig. 2). As observed with the prostacyclin metabolite, prostaglandin E_2 also was unchanged in the nonsensitive and sensitive-nonsensitive study groups; however in the sensitive-sensitive women levels were reduced an average of 26% (476 ± 173 to 351 ± 111 pg/ml, $p < 0.038$) after 1 week of low-dose aspirin therapy (Fig. 3). One week of low-dose aspirin therapy increased the ratio of 6-keto-prostaglandin $F_{1\alpha}$/thromboxane B_2 in the nonsensitive (0.57 ± 0.24 to 1.23 ± 0.48), sensitive-nonsensitive (0.87 ± 0.65 to 3.47 ± 3.09), and sensitive-sensitive (0.3 ± 0.46 to 2.17 ± 2.98) patients ($p < 0.001$). This difference was greatest in the angiotensin II patients who became nonsensitive after 1 week of low-dose aspirin therapy.

The effective pressor dose of angiotensin II and clinical outcomes for all groups are shown in Fig. 4. The mean effective pressor dose of angiotensin

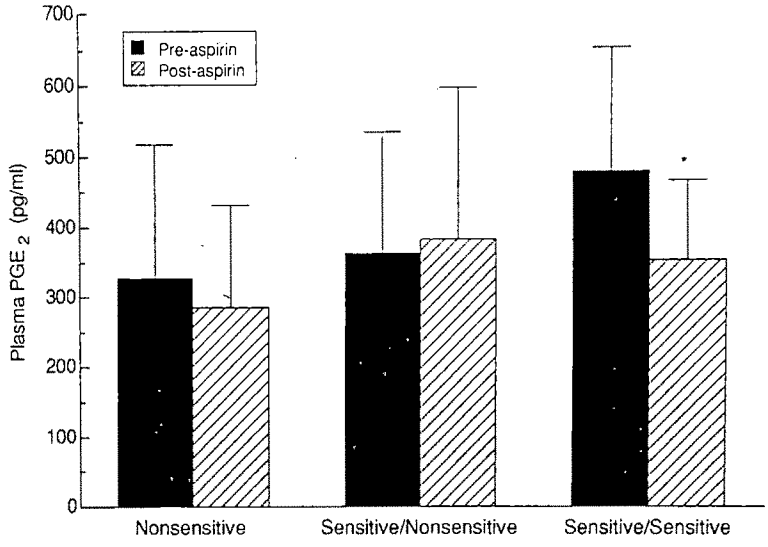


Fig. 3. Plasma concentrations of plasma prostaglandin E_2 in women who were sensitive or not sensitive to angiotensin II before and after low-dose aspirin therapy. *Asterisk*, $p < 0.038$ versus before aspirin.

II in the nonsensitive women was identical before and after 1 week of low-dose aspirin therapy (25.5 ± 10.6 and 25.5 = 10.6 ng/kg/min) (Fig. 4, *A*). Among these 18 patients, pregnancy-induced hypertension ultimately developed in seven (39%): four with mild disease (22%), two with preeclampsia (11%), and one with severe pregnancy-induced hypertension (6%). In the sensitive-nonsensitive group (Fig. 4, *B*), 6.3 ± 2.5 ng/kg/min was required initially to elicit the angiotensin II pressor response; 18.1 ± 7.9 ng/kg/min was required after aspirin therapy. Among these 11 patients, pregnancy-induced hypertension developed in four (36%): one with mild disease (9%), two with preeclampsia (18%), and one with severe pregnancy-induced hypertension (9%). In contrast, among the 11 women who were sensitive to the pressor effects of angiotensin II both before and after aspirin therapy (Fig. 4, *C*; 5.0 ± 2.5 and 4.7 ± 1.9 ng/kg/min, respectively) pregnancy-induced hypertension developed in all 11 women: four with mild disease (36%), two with preeclampsia (18%), and five with severe pregnancy-induced hypertension (45%). There was no difference in the occurrence of hypertension between the nonsensitive group (6/18) and the sensitive-nonsensitive group (4/11); however, there was a significant increase in the occurence of hypertensive complications in the sensitive-sensitive group (11/11) ($\chi^2 = 16.14$; $p < 0.001$) as compared with the other two groups.

Neonatal outcome data stratified by maternal angiotensin II pressor sensitivity are shown in Table II. There were no perinatal deaths and no birth weights below the 10th percentile for gestational age. Birth weights were lower among women who remained angiotensin II sensitive after aspirin therapy as compared with the other two groups ($p < 0.05$). The gestational age at delivery for women who were initially sensitive to angiotensin II was less than that of the nonsensitive women ($p < 0.05$).

We also evaluated whether the relationships described above for the serum and plasma eicosanoids in the three subpopulations of women also were observed when the data were stratified post hoc by the final clinical outcome (Table III). As expected, both serum and plasma thromboxane B_2 levels were decreased after 1 week of low-dose aspirin therapy in the patients who were normotensive and hypertensive at the end of gestation ($p < 0.01$). Although the plasma 6-keto-prostaglandin $F_{1\alpha}$ values were similar in the normotensive and hypertensive patients, aspirin decreased plasma 6-keto-prostaglandin $F_{1\alpha}$ concentrations 21% only in those women in whom hypertension subsequently developed ($p < 0.05$). The ratio of 6-keto-prostaglandin $F_{1\alpha}$/thromboxane B_2 was increased 181% and 198%, respectively in the normotensive and hypertensive study groups ($p < 0.01$). The decrease in prostacyclin metabolite was not specific since prostaglandin E_2 also was decreased by 25% only in the women who were subsequently diagnosed with hypertension ($p < 0.05$).

Comment

Low-dose aspirin therapy has been reported to decrease the occurrence of proteinuric hypertension and to have a beneficial effect on the clinical outcome in many but not all pregnancies.[11-13] It has been suggested that the control of normal vascular refractoriness to angiotensin II in pregnancy and the loss of this re-

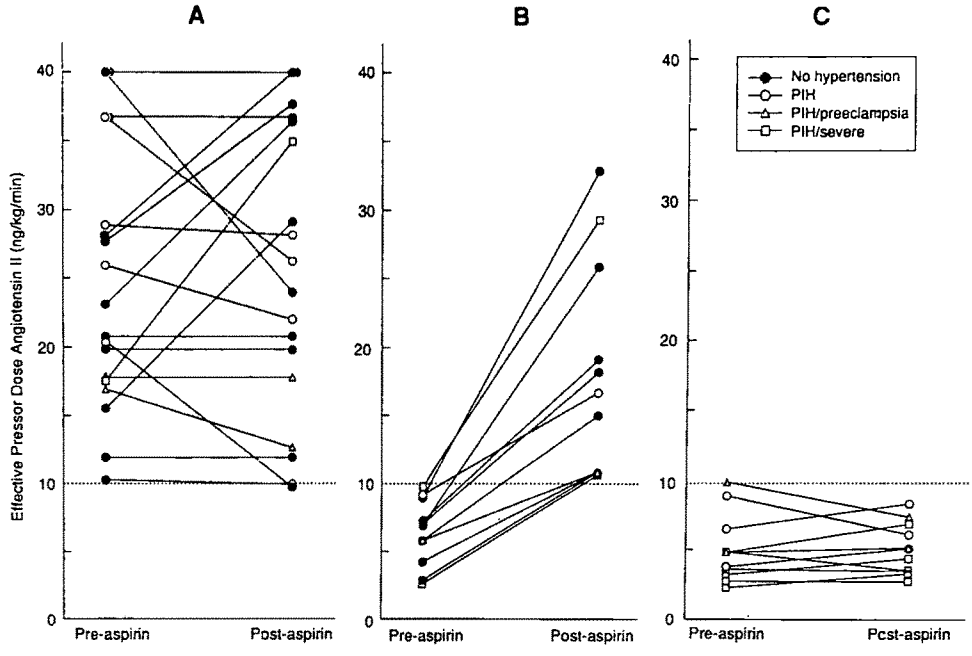


Fig. 4. Effective pressor dose and clinical outcome in women who were sensitive or not sensitive to angiotensin II before and after low-dose aspirin therapy. The occurrence of hypertension was **(A)** nonsensitive, 7 of 18; **(B)** sensitive-nonsensitive, 4 of 11; **(C)** sensitive-sensitive, 11 of 11 ($\chi^2 = 16.14$, $p < 0.001$).

fractoriness in women with pregnancy-induced hypertension may be the consequence of changes in the production of vascular prostaglandins[1-6] and, specifically, changes in the ratio of prostacyclin/thromboxane A_2.[9] In our initial report the effect of low-dose aspirin (81 mg/day) on the pressor response to angiotensin II was ascertained and related to levels of circulating eicosanoids.[14] The results of the present study are consistent with the view and are an extension of our prior observations,[14] with additional nulliparous patients, that there is a heterogeneity of pressor responses to infused angiotensin II during low-dose aspirin therapy. Moreover, unlike our previous study[14] we now present data that this heterogeneity is related to subsequent pregnancy outcome. In our previous report too few patients were studied either to evaluate pregnancy outcome or to stratify and report these data according to angiotensin II pressor sensitivity. One objective of the current study therefore was to expand the number of nulliparous women evaluated to determine clinical outcome. The second objective was to access the effect(s) of low-dose aspirin therapy in normal pregnant women who were refractory to angiotensin II, since there are clinical trials of low-dose aspirin therapy presently being conducted in large unselected populations.

The clinical outcome for nulliparous women who were initially sensitive to the pressor effects of angiotensin II and remained sensitive was not improved by low-dose aspirin treatment (Fig. 4, *C*). Specifically, 100% of the women who were sensitive to angiotensin II both before and after 1 week of low-dose aspirin subsequently had hypertensive complications. These women also had earlier delivery, and thus their neonates had lower birth weights. In addition to the differences in the pharamcologic and physiologic responses and clinical outcome of this subpopulation of women as compared with the two other groups, the eicosanoid responses to low-dose aspirin therapy also were different. As expected, plasma and serum thromboxane B_2 levels were inhibited in all three groups, demonstrating therapeutic compliance; however, only in the sensitive-sensitive group were both plasma 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin E_2 values also decreased. Inhibition of 6-keto-prostaglandin $F_{1\alpha}$ production has been reported by others.[13, 18] The observation that the prostacyclin metabolite and prostaglandin E_2 were decreased significantly only in the women who remained angiotensin II sensitive after 1 week of low-dose aspirin therapy might be explained by differences in the metabolic clearance rate and/or the apparent volume of distribution of the aspirin. However, our observation that plasma thromboxane B_2 was decreased to a similar extent in all three groups argues against the hypothesis that changes in the metabolic clearance rate might account for this phenomenon. Evidence against there being a difference in the apparent volume of distribution is obtained from the observation that, although the women in the sensitive-sensitive

Table II. Neonatal outcome stratified by maternal angiotensin II sensitivity

	*Nonsensitive**	*Sensitive-nonsensitive*	*Sensitive-sensitive**
Gestational age at delivery (wk)	39.5 ± 1.3[a]	37.2 ± 3.3[b]	35.6 ± 2.6[b]
Birth weight (gm)	3196 ± 593[a]	2831 ± 778[a]	2183 ± 490[b]

Values are means ± SD. Means with different superscripts (*a, b*) are different ($p < 0.05$).
*There was one set of twins in each of these groups.

Table III. Eicosanoid concentrations after 1 week of low-dose aspirin therapy in serum and plasma from pregnant women who became normotensive and hypertensive

	Normotensive (n = 16)		*Hypertensive (n = 18)*	
	Control	*Aspirin*	*Control*	*Aspirin*
Serum thromboxane B_2	1430 ± 1161	103 ± 72*	1307 ± 1266	135 ± 165*
Plasma thromboxane B_2	338 ± 165	132 ± 59*	288 ± 187	102 ± 55*
6-Keto-prostaglandin $F_{1\alpha}$	175 ± 113	153 ± 80	202 ± 111	154 ± 32†
Prostaglandin E_2	360 ± 173	367 ± 186	390 ± 203	294 ± 130†
6-Keto-prostaglandin $F_{1\alpha}$/thromboxane B_2	0.52 ± 0.22	1.46 ± 1.42‡	0.88 ± 0.53	2.62 ± 0.85‡

Values are means ± SD.
*$p < 0.001$, versus control.
†$p < 0.05$, versus control.
‡$p < 0.01$, versus control.

treatment group weighed 18% to 23% more than those in the other two groups, the plasma thromboxane B_2 levels were decreased similarly, and only in the heavier group were there decreases in 6-keto-prostaglandin $F_{1\alpha}$ as well as prostaglandin E_2. An alternative hypothesis is that the cyclooxygenase enzyme sensitivity to aspirin of the women in the sensitive-sensitive group is greater than that in the other two groups. It is also possible that the ability of the tissues to synthesize new cyclooxygenase enzyme after the irreversible inhibition by aspirin is different in the women who remained sensitive to angiotensin II.

After 1 week of low-dose aspirin therapy, 50% of sensitive women became refractory to the pressor effects of infused angiotensin II, i.e., the sensitive-nonsensitive subgroup (Fig. 4, *B*). Although inhibition of serum and plasma thromboxane B_2 was similar in all three groups, plasma levels of 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin E_2 were unaltered after 1 week of low-dose aspirin. Furthermore, those angiotensin II–sensitive patients who became refractory to the pressor effects of angiotensin II after 1 week of low-dose aspirin therapy had improved clinical outcomes; that is, their pregnancies were less likely to be complicated by hypertension and they were delivered nearer to term. Although the occurrence of hypertension in this group, four of 11 (36%), was significantly less than that in the nonresponders (sensitive-sensitive), it was higher than expected from previous reports of similarly selected patients[12] or a population mixed with multiparous women.[13] The reason(s) for this is unclear but may be explained in part by the fact that low-dose aspirin therapy in the current study was instituted later in pregnancy in these women with positive rollover test results and in some women only after the onset of mild, transitory hypertension. Other factors such as the dose of aspirin (81 vs 60 mg),[12] population differences, a combination of the above, or as yet unidentified factors might also have contributed to this result.

There was one notable exception to the above, a 15-year-old Hispanic woman who had been admitted for in-hospital bed rest because of transient hypertension and subsequently left against medical advice. Blood pressure had normalized (107/56 mm Hg) before her first angiotensin II infusion; the effective pressor dose was 9.8 ng/kg/min at 31 weeks' gestation and increased to 29.2 ng/kg/min after 1 week of low-dose aspirin therapy. While in the hospital and receiving aspirin therapy, normal blood pressure readings were maintained. She left the hospital at 35 weeks' gestation and discontinued aspirin. She returned 15 days later in a postictal condition with a blood pressure of 170/120 mm Hg and proteinuria (2+). The diagnosis of eclampsia was made and she had one more seizure before delivery. The effect of discontinuing aspirin therapy on eicosanoid production and clinical outcome is unknown, and unfortunately blood specimens were not obtained after her return to the hospital.

A relatively high occurrence of hypertensive complications (39%) was observed among the women who

were outpatients without evidence of incipient hypertension. Although these women were enrolled in the study after a positive rollover test result, they were refractory to infused angiotensin II. Although there was no change in the effective pressor dose of angiotensin II among these women as a group (Fig, 4, *A*), those who became more sensitive to the pressor effects of angiotensin II after aspirin therapy but still required ≥10 ng/min/kg had an 80% occurrence (4/5) of hypertension. There was one exception worth noting. This woman had an effective pressor dose of angiotensin II that increased from 17.5 to 35 ng/kg/min after 1 week of low-dose apsirin; despite this increased refractoriness to infused angiotensin II, severe preeclamspia developed. It is unclear what differentiates these women from the others in this group. They may represent instances of noncompliance after the last study period. Since pregnancy-induced hypertension developed in seven of 18 (39%) initially nonsensitive patients, an observation not anticipated, it is possible that there may be some as yet undescribed adverse effects from low-dose aspirin treatment in normal gravid women. Nevertheless, this group of women did have significantly longer overall gestations than those initially sensitive to angiotensin II.

In addition to the prehoc stratification of the eicosanoid data on the basis of angiotensin II sensitivity, we also performed a post hoc stratification of the data on the basis of whether these women became hypertensive by the end of pregnancy (Table III). We observed similar relationships as noted earlier, i.e., both groups had similar and dramatic falls in serum and plasma thromboxane B_2 levels, confirming therapeutic compliance; however, only in those women in whom hypertension subsequently developed did significant decreases in 6-keto-prostaglandin $F_{1\alpha}$ (21%) and prostaglandin E_2 (25%) concentrations occur. The 6-keto-prostaglandin $F_{1\alpha}$/thromboxane B_2 ratio has been suggested to be an important factor in, or marker of, pregnancy-induced hypertension.[1-6, 9, 14] When we examined the changes in this ratio in the plasma of women in the three study groups on the basis of angiotensin II sensitivity before and during low-dose aspirin therapy, it was increased 116% in the nonsensitive, 299% in the sensitive-nonsensitive, and 171% in the sensitive-sensitive patients after 1 week of low-dose aspirin therapy. This rise was greatest in those women who became nonsensitive after 1 week of low-dose aspirin therapy whether they did or did not subsequently become hypertensive. However, the relative increase in the ratio after 1 week of low-dose aspirin therapy was similar when stratified according to the subsequent development of hypertension, i.e., normotensive (181%) or hypertensive (198%) (Table III). Thus this greater effect of low-dose aspirin therapy to increase this ratio in the sensitive-nonsensitive group appeared to occur concomitantly with the changes in the effective pressor dose of angiotensin II rather than as a predictor of the future hypertensive complications.

The relationship observed in this study among differences in vascular reactivity, different patterns of prostaglandin response to low-dose apsirin, and clinical outcome is suggestive of a related, underlying pathophysiologic phenomenon. An attractive single hypothesis is that a metabolic and/or an enzymatic defect renders some women incapable of maintaining an appropriate balance of factors that act to modulate vascular reactivity. Although prostaglandins may play a role in the pathogenesis of pregnancy-induced hypertension,[4, 19] other factors may work alone or in combination with prostaglandins to contribute to the maintenance of normal vascular reactivity. Metabolic or enzymatic defects are often genetically determined in an autosomal recessive manner, and several authors[20-22] have suggested that the predisposition for preeclampsia may reflect this type of defect. Whether the associations observed in this study are due to a single enzyme defect or a more complex situation remains to be ascertained. Our observations regarding low-dose aspirin therapy further support the concept that this agent is not "the answer" for the prevention of preeclampsia in all women, at least in the setting and dosage used in this study.

We thank Sheri Teriot, Lucy Dodd, Tammie Plumlee, and Stephanie Higgins for their technical assistance and the house officers in Obstetrics and Gynecology at Parkland Memorial Hospital for their assistance in the clinical management of the women described in this study. We also wish to thank Ciba-Geigy for their generous gift of the angiotensin II amide used in this investigation.

REFERENCES

1. Abdul-Karim R, Assali NS. Pressor response to angiotensin in pregnant and nonpregnant women. AM J OBSTET GYNECOL 1961;82:246.
2. Chesley LC, Talledo E, Bohler CS, Zuspan FP. Vascular reactivity to angiotensin II and norepinephrine in pregnant and nonpregnant women. AM J OBSTET GYNECOL 1965;91:837.
3. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest 1973;52:2682.
4. Everett RB, Worley RJ, MacDonald PC, Gant NF. Effect of prostaglandin synthetase inhibitors on pressor response to angiotensin II in human pregnancy. J Clin Endocrinol Metab 1978;46:1007.
5. Magness RR, Osei-Boaten K, Mitchell MD, Rosenfeld CR. In vitro prostacyclin production by ovine uterine and systemic arteries: effects of angictensin II. J Clin Invest 1985;76:2206.
6. Magness RR, Mitchell MD, Rosenfeld CR. Uteroplacental production of eicosanoids in ovine pregnancy. Prostaglandins 1990;39:75.
7. Fitzgerald DJ, Entman SS, Mulloy K, Fitzgerald GA. Decreased prostacyclin biosynthesis preceding the clinical

manifestation of pregnancy-induced hypertension. Circulation 1987;75:956.
8. Goodman RP, Killam AP, Brash AR, Branch RA. Prostacyclin production during pregnancy: comparison of production during normal pregnancy and pregnancy complicated by hypertension. AM J OBSTET GYNECOL 1982;142:817.
9. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. AM J OBSTET GYNECOL 1985;152:335.
10. Fitzgerald DJ, Mayo G, Catella F, Entmann S, Fitzgerald GA. Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets. AM J OBSTET GYNECOL 1987;157:325.
11. Beaufils M, Uzan S, Donsimoni R, Colau JC. Prevention of pre-eclampsia by early antiplatelet therapy. Lancet 1985;1:840.
12. Wallenburg HCS, Dekker GA, Makovitz JW, Rotmans P. Low-dose aspirin prevents pregnancy-induced hypertension and preeclampsia in angiotensin-sensitive primigravidae. Lancet 1986;1:1.
13. Schiff E, Peleg E, Goldenberg M, et al. The use of aspirin to prevent pregnancy-induced hypertension and lower the ratio of thromboxane A_2 to prostacyclin in relatively high risk pregnancies. N Engl J Med 1989;321:351.
14. Spitz B, Magness RR, Cox SM, Brown CEL, Rosenfeld CR, Gant NF. Low-dose aspirin. I. Effect on angiotensin II pressor responses and blood prostaglandin concentrations in pregnant women sensitive to angiotensin II. AM J OBSTET GYNECOL 1988;159:1035.
15. Sanchez-Ramos L, O'Sullivan M, Garrido-Calderon J. Effect of low-dose aspirin on angiotensin II pressor response in human pregnancy. AM J OBSTET GYNECOL 1987;156:193.
16. Caruso A, Ferrazzani S, DeCarolis S, Lanzone A, Mancuso S. Effects of low-dose aspirin on vascular sensitivity to angiotensin II and on 24 hours arterial blood pressure in pregnancy. Clin Exp Hypertens Hypertens Pregnancy 1988; B7:171.
17. Cunningham FG, MacDonald PC, Gant NF. Hypertensive disorders in pregnancy. In: Williams' obstetrics. 18th ed. Norwalk, Connecticut: Appleton & Lange, 1989:653.
18. Sibai BM, Mirro R, Chesney CM, Leffler C. Low-dose aspirin in pregnancy. Obstet Gynecol 1989;74:551.
19. Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol 1988;71:122.
20. Chesley LC. Hypertension in pregnancy: definitions, familial factor, and remote prognosis. Kidney Int 1980;18 (suppl):234.
21. Sutherland A, Cooper DW, Howie PW, Liston WA, MacGillivray I. The incidence of severe pre-eclampsia amongst mothers and mothers-in-law of pre-eclamptics and controls. Br J Obstet Gynaecol 1981;88:785.
22. Cooper DW, Liston WA. Genetic control of severe pre-eclampsia. J Med Genet 1979;16:409.

Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat

William C. Mabie, MD, Robert A. Ahokas, PhD, and Baha M. Sibai, MD

Memphis, Tennessee

The chronic effects of captopril on maternal hemodynamics and organ perfusion were investigated in 10 untreated and 10 captopril-treated pregnant spontaneously hypertensive rats by means of the radioactive-labeled microsphere technique. The normal decrease in blood pressure during gestation was prevented by reduction of litter size to two conceptuses on day 7 of gestation. Captopril (≈10 mg/kg/day) or drug vehicle (50% ethyl alcohol) was administered intraperitoneally by an osmotic pump from day 7 to 21. At term mean arterial pressure was 23% lower in the captopril-treated group as the result of a 29% decrease in total peripheral resistance without a significant change in cardiac output. The decrease in total peripheral resistance was primarily caused by a decline in splanchnic and skin resistances. Maternal organ and uteroplacental perfusion were not significantly altered. We conclude that administration of captopril during the last 2 weeks of pregnancy in the hypertensive rat effectively lowers maternal blood pressure without adverse effects on organ and uteroplacental perfusion. (AM J OBSTET GYNECOL 1990;163:1861-7.)

Key words: Captopril, total peripheral resistance, placental perfusion, spontaneously hypertensive rat

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Tennessee-Memphis.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: William C. Mabie, MD, Department of Obstetrics and Gynecology, University of Tennessee-Memphis, 853 Jefferson Ave., Memphis, TN 38103.
6/6/24774

In 1981 captopril was approved by the Food and Drug Administration for use as an antihypertensive agent in the United States.[1] Since then two other angiotensin-converting enzyme inhibitors, enalapril and lisinopril, have been approved and at least 14 other such inhibitors are under development.[2] Because of

their efficacy and low side-effect profile angiotensin-converting enzyme inhibitors are widely used as first-line therapy for hypertension.

The mechanism of action of the angiotensin-converting enzyme inhibitors is not completely understood, but it is thought that they induce vasodilatation by inhibiting angiotensin-converting enzyme, the enzyme that converts angiotensin I to angiotensin II. In addition to being a potent vasoconstrictor, angiotensin II stimulates aldosterone secretion and increases sympathetic outflow centrally. Because angiotensin-converting enzyme is identical to kininase II, which inactivates bradykinin, an additional antihypertensive effect may result from increased bradykinin levels. Finally, certain angiotensin-converting enzyme inhibitors increase synthesis of the vasodilating prostaglandins E_2 and I_2.[2-4] Accumulating evidence indicates that the major site of angiotensin II production is not in blood but in tissues (e.g., vascular, cardiac, renal, and brain). The decrease in blood pressure correlates better with the degree of inhibition of the vascular tissue renin-angiotensin system than with the circulating renin-angiotensin system.[2]

As with any antihypertensive drug, the primary concerns about the use of captopril in pregnancy are the possible adverse effects on placental perfusion and the risk of congenital malformations. Captopril caused fetal death in rabbits, apparently by reduction of uteroplacental perfusion.[5] It caused maternal and fetal hypotension and stillbirth in sheep.[6] Although results are conflicting, congenital malformations and other fetal complications have been associated with its use in human pregnancy.[7-9] Therefore it has been recommended that captopril not be used during pregnancy.[8, 10]

The spontaneously hypertensive rat is widely accepted as a model of human essential hypertension. However, even in this genetically hypertensive animal, pregnancy has a profound blood pressure–lowering effect. During the last week of gestation blood pressure progressively decreases and usually reaches normotensive levels by term. We have recently shown that the magnitude of the decrease in blood pressure in the pregnant spontaneously hypertensive rat is directly proportional to litter size.[11] The resistances of virtually all the maternal organ vascular beds, including the uterus and placentas, at term are inversely related to litter size. Thus reducing litter size to one or two conceptuses shortly after implantation prevents the normal decrease in blood pressure, producing a model of essential hypertension during pregnancy. The purpose of this study was to evaluate the effects of lowering maternal blood pressure with captopril during the last 2 weeks of pregnancy on maternal and uteroplacental hemodynamics at term in the hypertensive, pregnant rat.

Material and methods

This research conforms with the "Guiding Principles in the Care and Use of Laboratory Animals" approved by the Council of the American Physiological Society and with federal laws and regulations. The protocol used was approved by the University of Tennessee, Memphis, Animal Care and Use Committee.

Virgin female spontaneously hypertensive rats (10 to 12 weeks of age) were purchased from Harlan/Sprague-Dawley, Inc. (Indianapolis). They were housed four per cage in a temperature-controlled room (23° ± 1° C) with lights on from 7 AM to 7 PM and were fed Purina Laboratory Rodent Chow (Ralston Purina, St. Louis) as desired with tap water to drink. For breeding, the females were housed 1 : 1 with mature male spontaneously hypertensive rats. Vaginal smears were checked daily in the morning for the presence of spermatozoa. The day vaginal smears were sperm positive was designated day 0 of pregnancy and the timed pregnant rats were housed singly throughout pregnancy.

Systolic blood pressure was measured by tail-cuff plethysmography (IITC, Inc., Woodland Hills, Calif.) and was monitored every other day throughout gestation. For these measurements the rats were prewarmed to 30° C for 15 to 20 minutes in an incubator. They were then placed in a Plexiglas restraining cage and an inflatable cuff with a photoelectric sensor was placed around the base of the tail. The cuff was then inflated to a pressure of 250 mm Hg. The pressure was gradually released until a pulse was detected (systolic blood pressure). The mean of 3 to 5 determinations was recorded for each day.

On day 7 of gestation a laparotomy was performed with the rat under light methoxyflurane anesthesia and litter size was reduced to two (one in each uterine horn) by aspiration of embryos through small incisions in the antimesometrial uterine wall. The uterine incisions were then closed with sutures. A sterile osmotic minipump (model 2001, Alza Corp., Palo Alto, Calif.) filled with either a sterile solution of captopril (200 mg/ml) or drug vehicle (50% ethyl alcohol) was placed in the abdominal cavity to administer the contents intraperitoneally. Infusion rate was 0.5 μl/hr (2.4 mg of captopril per day). This rate of infusion resulted in a dosage of ~10 to 12 mg/kg/day. The abdominal incisions were closed, and the rats were injected with penicillin G (30,000 U/kg intramuscularly) and returned to their cages for the duration of pregnancy.

On postmating day 21 (the day before delivery), mean arterial pressure, cardiac output, and organ blood flows were measured with the radioactive-labeled microsphere technique described previously.[12-14] Mean arterial pressure, cardiac output, and organ blood flows

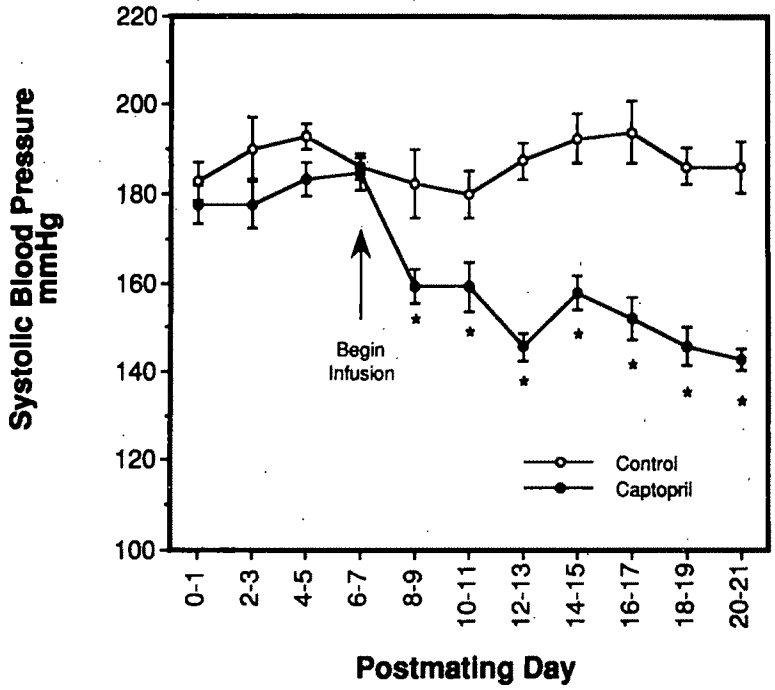


Fig. 1. Changes in systolic blood pressure during gestation in control and captopril-treated spontaneously hypertensive rats. Osmotic pump for intraperitoneal drug administration was implanted on day 7. Data are plotted as mean ± SEM. *Asterisk* indicates significant difference between groups ($p < 0.05$).

were measured after a minimum 2-hour anesthesia recovery period.

The data were expressed as the mean ± SEM. Systolic blood pressure data was analyzed by two-way analysis of variance. Student *t* test was used for comparison of mean arterial pressure, cardiac output, total peripheral resistance, organ flows, and organ vascular resistances between groups. The 95% confidence level was accepted as statistically significant for all analyses.

Results

Fig. 1 depicts the mean systolic blood pressure measured on alternate days during gestation for the untreated and captopril-treated rats. On day 0, mean systolic blood pressure in the two groups was not different. On day 7, the day treatment was started, mean systolic blood pressure was 186 ± 3 mm Hg and 185 ± 4 mm Hg in the untreated and captopril-treated groups, respectively. Captopril produced a significant reduction in mean systolic blood pressure that persisted throughout gestation beginning on the first day or two after pump implantation. On day 21, mean systolic blood pressure was 186 ± 6 mm Hg and 143 ± 2 mm Hg in the untreated and captopril-treated rats, respectively.

Table I shows the total body, maternal organ, and fetal and placental weights of the untreated and captopril-treated rats. Net maternal weight, but not mean fetal or placental weight, was significantly lower in the captopril-treated compared with the untreated rats. This was primarily a result of lower carcass, skin, and liver weights. Captopril treatment may have resulted in reduced food consumption, although this factor was not measured. Nine of the control rats had two fetuses at term compared with six of the captopril-treated rats, a nonsignificant difference. One rat in the control group and four in the captopril-treated group aborted one conceptus and carried one to term. There was no decaying tissue found in the uteri of the rats with only one fetus that would suggest recent fetal death, and all living fetuses appeared structurally normal. There was no significant difference in mean fetal or placental weight between the two groups.

The effects of captopril on maternal hemodynamics at term are summarized in Fig. 2. Mean arterial pressure was 23% lower in the captopril-treated group than in the control group (130.2 ± 4.7 mm Hg vs 169.0 ± 3.6 mm Hg, $p < 0.01$). Maternal cardiac index was unchanged but total peripheral resistance of the captopril-treated rats was 29% lower than that of the untreated rats (4.68 ± 0.38 vs 3.33 ± 0.30 mm Hg/ml/min/100/gm, $p < 0.02$).

There was a nearly significant increase ($p = 0.06$) in apparent lung blood flow (1.6 ± 0.3% vs 5.2 ± 1.8%

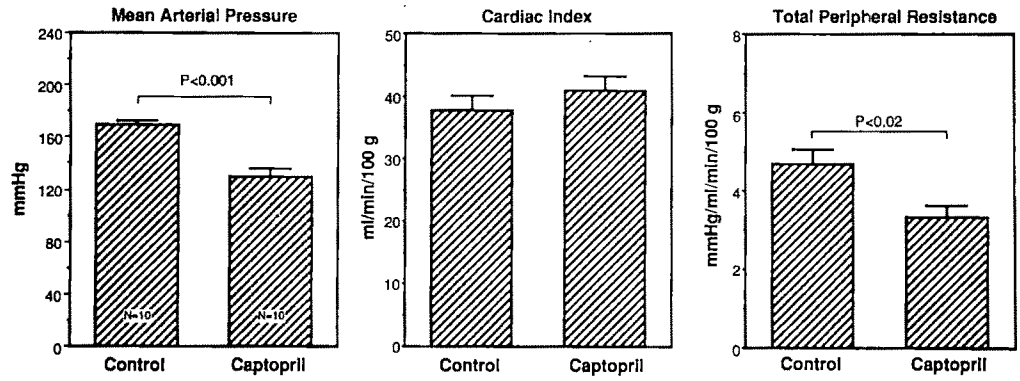


Fig. 2. Mean arterial pressure, cardiac index, and total peripheral resistance at term in 10 control and 10 captopril-treated (≈10 mg/kg/day) spontaneously hypertensive rats.

Table I. Total body, maternal organ, and fetal and placental weights of untreated and captopril-treated rats

	Control	*Captopril*	*Significance*
Total body weight, day 0 (gm)	197.2 ± 3.5	211.0 ± 7.0	
Total body weight, day 21 (gm)	251.2 ± 7.0	263.6 ± 5.0	$p \leq 0.10$
Net maternal weight, day 21 (gm)	207.8 ± 6.0	189.6 ± 3.5	$p \leq 0.02$
Carcass (gm)	142.33 ± 4.74	129.63 ± 2.49	$p \leq 0.05$
Skin (gm)	34.45 ± 0.92	32.09 ± 0.72	$p \leq 0.05$
Brain (gm)	1.82 ± 0.03	1.80 ± 0.03	
Heart (gm)	1.06 ± 0.04	0.95 ± 0.06	$p < 0.10$
Lungs (gm)	1.21 ± 0.04	1.17 ± 0.03	
Stomach (gm)	1.30 ± 0.05	1.25 ± 0.03	
Small intestine (gm)	4.95 ± 0.18	4.81 ± 0.19	
Large intestine (gm)	3.32 ± 0.11	3.21 ± 0.11	
Liver (gm)	11.07 ± 0.34	10.15 ± 0.28	$p < 0.05$
Spleen (gm)	0.45 ± 0.18	0.48 ± 0.03	
Pancreas (gm)	0.67 ± 0.07	0.62 ± 0.05	
Kidneys (gm)	1.56 ± 0.17	1.53 ± 0.05	
Adrenal glands (gm)	0.08 ± 0.01	0.08 ± 0.01	
Ovaries (gm)	0.11 ± 0.01	0.11 ± 0.01	
Uterus (gm)	2.15 ± 0.12	1.71 ± 0.11	$p < 0.05$
Litter size	19/20	16/20	
Mean fetal weight (gm)	4.72 ± 0.22	4.65 ± 0.26	
Mean placental weight (gm)	0.58 ± 0.03	0.59 ± 0.09	

of cardiac output) in the untreated and captopril-treated groups, respectively. This may have been a result of the opening of systemic arteriovenous shunts by captopril.

Fig. 3 illustrates the splanchnic, skin, and remaining carcass blood flows and vascular resistances of untreated and captopril-treated rats. Captopril produced significant reductions in splanchnic and skin vascular resistances but had no significant affect on splanchnic, skin, or carcass blood flows.

Fig. 4 summarizes the cardiac, renal, adrenal, and reproductive organ blood flows and resistances in untreated and captopril-treated rats. There were no statistically significant differences between groups in either blood flow or resistance values.

Comment

The traditional concept of the renin-angiotensin system is that of a circulation-borne endocrine system in which angiotensinogen is produced by the liver, renin by the kidney, and angiotensin-converting enzyme by the lung. The product of this biochemical cascade, angiotensin II, acts on specific receptors in multiple target organs. However, recent data demonstrate that renin and angiotensin are synthesized locally in many tissues (e.g., blood vessels, heart, lungs, kidneys, adrenals, and brain). The emerging concept is that angiotensin II produced locally at vascular tissue sites by an endogenous renin-angiotensin system may play an important role in the tonic control of vascular resistance. This concept implies that local angiotensin II concentrations

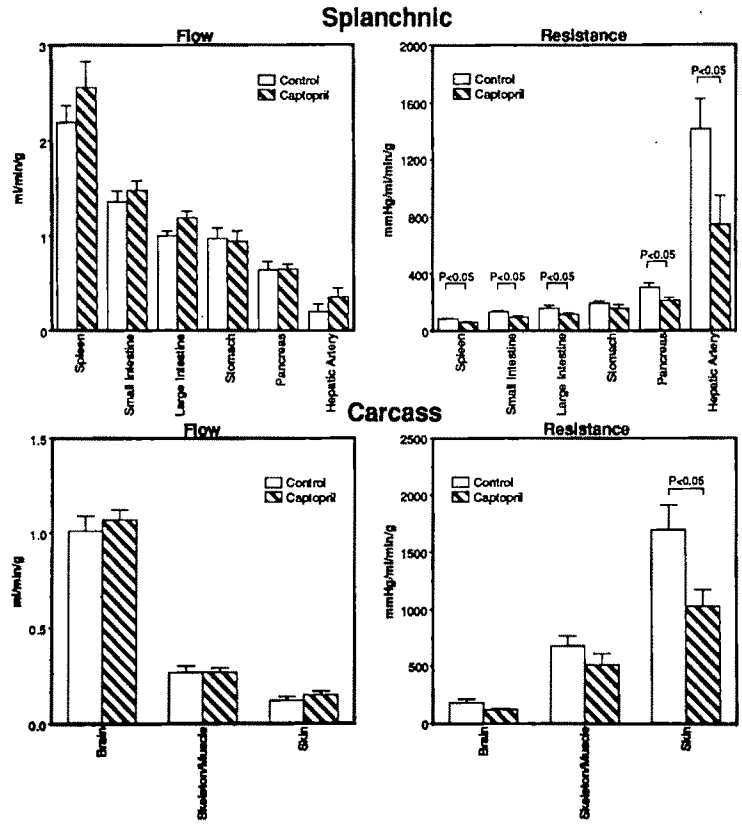


Fig. 3. Splanchnic and carcass blood flows and vascular resistances at term in 10 control and 10 captopril-treated spontaneously hypertensive rats.

may exceed plasma levels and helps to explain why angiotensin-converting enzyme inhibitors lower vascular resistance in normal and low renin hypertension, why nephrectomized rats continue to produce angiotensin II, and why the duration of antihypertensive response to angiotensin-converting enzyme inhibitors far exceeds the duration of serum angiotensin-converting enzyme inhibition.[3]

The results of this study show that chronic intraperitoneal captopril (~10 mg/kg/day) lowered blood pressure by decreasing total peripheral resistance without affecting cardiac output. The decrease in total peripheral resistance was primarily a result of a significant decline in splanchnic and skin resistances, although small decrements in resistance occurred in many other organs including the kidney, adrenal, uterus, placenta, ovary, skeletal muscle, and brain. Because blood flow did not increase significantly in any organ, perfusion pressure must have fallen in parallel with resistance.

These results differ from our previous studies of other antihypertensive drugs in the hypertensive, pregnant spontaneously hypertensive rat. Short-term administration of hydralazine and nifedipine produced generalized vasodilatation except in the skin and in many of the splanchnic organs.[12, 13] With labetalol the antihypertensive effect was primarily the result of a decline in cardiac output, but small decrements in resistance occurred in many regions except the carcass and splanchnic region.[14] Although specific reasons for the different regional vascular effects are not precisely known, they are probably related to the different mechanisms of drug action and to distribution of receptors blocked by the various drugs. The fact that captopril had a more pronounced effect on skin and splanchnic organ resistance suggests that these vascular beds may be more dependent on the renin-angiotensin system for regulation of their tone than other vascular beds in the body. However, the other vascular beds may also be constricted by increased sympathetic nervous activity as a result of a baroreflex compensatory response to the decrease in blood pressure induced by dilatation in the skin and splanchnic beds. In our experience, the skin and splanchnic regions receive 20% to 30% of total cardiac output in the conscious, term-pregnant spontaneously hypertensive rat (unpublished data). Sizable falls in resistance in these large vascular beds could

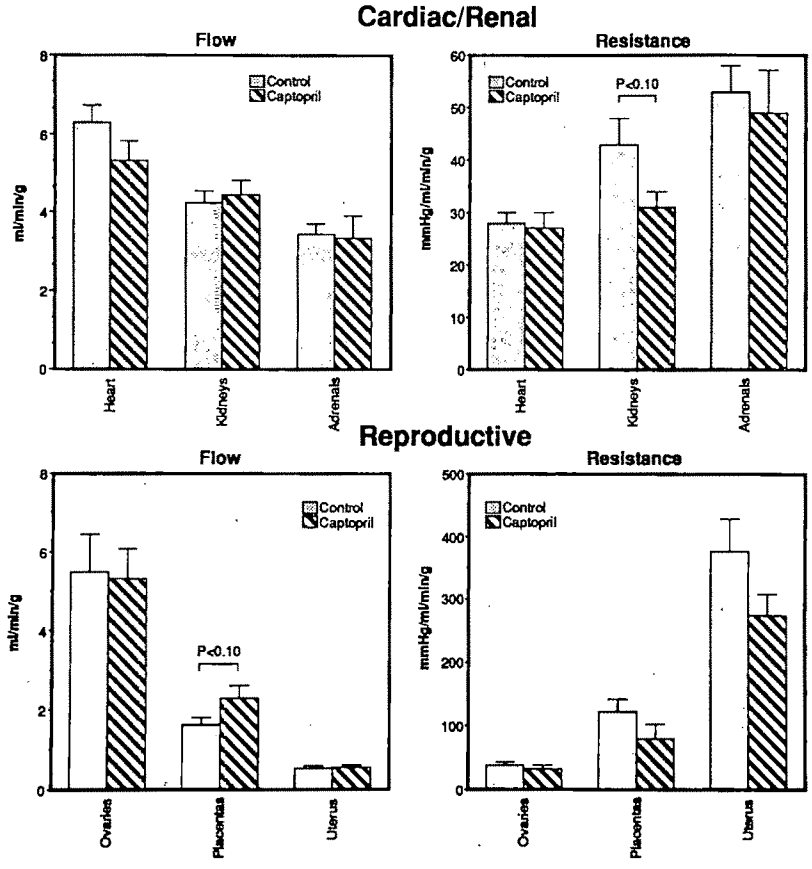


Fig. 4. Cardiac/renal and reproductive organ blood flows and resistances at term in 10 control and 10 captopril-treated spontaneously hypertensive rats.

conceivably reduce blood pressure enough to trigger baroreflex compensatory vasoconstriction, thus offsetting generalized vasodilatation produced by captopril.

Koike et al.[15] administered captopril orally once daily at doses of 10 mg/kg and 30 mg/kg for 6 weeks to male spontaneously hypertensive rats. The decrease in systolic blood pressure was less than that observed in our study even at the 30 mg/kg dose. By use of the radioactive-labeled microsphere technique, cardiac output was found to increase in a dose-dependent manner and reached statistical significance in the high-dose group, whereas total peripheral resistance decreased. Cerebral and renal blood flow increased dose-dependently, whereas flow to other organs was not significantly affected. Antonaccio et al.[16] reported that oral captopril (30 mg/kg) increased blood flow to all organs examined in spontaneously hypertensive rats, and the effect was statistically significant for the splanchnic area. We do not know why our results differ from these results, but it may be because of different routes of drug administration; because of constant, low-level exposure to captopril rather than bolus, high concentrations of the drug; or because we used pregnant female spontaneously hypertensive rats.

In this model use of captopril produced no adverse effect on uteroplacental blood flow. These findings are in contrast with the work of Ferris and Weir,[5] who noted in normotensive pregnant rabbits that acute captopril administration (5 mg/kg intravenously) reduced uterine and placental blood flow. They speculated that blockade of angiotensin II synthesis with a resultant decrease in uterine prostaglandin E_2 synthesis may have been the cause. In chronic studies with rabbits, captopril (2.5 or 5 mg subcutaneously daily) caused a marked decrease in fetal survival. Similarly, Broughton Pipkin et al.[6] found that administration of captopril (2.8 to 3.5 mg/kg intravenously) was associated with a much increased stillbirth rate in both sheep and rabbits; however, enalapril (1 or 2 mg/kg intravenously), which apparently does not cross the sheep placenta, was associated with a lower stillbirth rate.[17] The loss of four conceptuses in our captopril-treated group compared with only one conceptus in the control group, although not statistically significant, is at least consonant with the observations made in rabbits and sheep that use of captopril is associated with increased pregnancy wastage. The loss of conceptuses in both groups of rats apparently occurred early in gestation and was probably re-

lated to surgical manipulation of the uterus because there was no decaying tissue or other evidence of a second pregnancy found in any of the rats with a single fetus. Our previous experience[11] has been that manipulation of the uterus to adjust litter size occasionally causes one or more of the adjacent conceptuses to abort. The fact that the remaining pups were normal in weight and development supports this explanation. If captopril had induced uterine ischemia, the surviving fetus and placenta would be expected to be growth retarded. This does not conclusively rule out the possibility that captopril caused the abortions, and further study is needed in this area.

In human pregnancy angiotensin-converting enzyme inhibitor therapy has been associated with several fetal and maternal complications including hypotension, growth retardation, oligohydramnios, anuria, renal failure, congenital malformations, stillbirth, and neonatal death.[8-10] However, more favorable results have been reported. Coen et al.[18] treated a patient with chronic glomerulonephritis throughout a twin pregnancy with a combination of captopril, hydralazine, and metoprolol. At term she was delivered of growth-retarded twins whose subsequent course to 10 months of age was unremarkable. Mochizuki et al.[19] reported good results with captopril as part of combination therapy in late pregnancy in three patients. Kreft-Jais et al.[20] reported the results of a French survey of pregnancies exposed to angiotensin-converting enzyme inhibitors between June 1985 and December 1986. Twenty-two women received captopril and nine received enalapril. Most women had renal disease or chronic essential hypertension. Two stillbirths occurred in the captopril group and one in the enalapril group. Preterm delivery occurred in 11 women who were given captopril and in one given enalapril. Six of 26 live-born babies were growth retarded.

In conclusion, chronic administration of captopril lowered blood pressure in the pregnant, hypertensive rat by decreasing total peripheral resistance without affecting cardiac output. Uteroplacental perfusion was unchanged, and no fetal growth retardation or congenital anomalies were noted. Nevertheless, because of conflicting animal data, potential risks to the fetus, and the availability of alternate drugs, the use of captopril in pregnant women should be avoided until further animal studies and human drug trials prove its safety.

We thank Ed B. Cannon for his laboratory technical assistance.

REFERENCES

1. Bakris GL, Frolich ED. The evolution of antihypertensive therapy: an overview of four decades of experience. J Am Coll Cardiol 1989;14:1595-1608.
2. Williams GH. Converting-enzyme inhibitors in the treatment of hypertension. N Engl J Med 1988;319:1517-25.
3. Dzau VJ. Implications of local angiotensin production in cardiovascular physiology and pharmacology. Am J Cardiol 1987;57:59A-65A.
4. Brogden RN, Todd PA, Sorkin EM. Captopril: an update on its pharmacodynamic and pharmocokinetic properties, and therapeutic use in hypertension and congestive heart failure. Drugs 1988;36:540-600.
5. Ferris TF, Weir EK. Effect of captopril on uterine blood flow and prostaglandin E synthesis in the pregnant rabbit. J Clin Invest 1983;71:809-15.
6. Broughton Pipkin F, Symonds EM, Turner SR. The effect of captopril (SQ14, 225) upon mother and fetus in the chronically cannulated ewe and in the pregnant rabbit. J Physiol 1982;323:415-22.
7. Duminy PC, Burger P du T. Fetal abnormality associated with the use of captopril during pregnancy. S Afr Med J 1981;60:805.
8. Broughton Pipkin F, Baker PN, Symonds EM. ACE inhibitors in pregnancy. Lancet 1989;2:96-7.
9. Guignard JP, Gouyon JB. Adverse effect of drugs on the immature kidney. Biol Neonate 1988;53:243-52.
10. Lindheimer MD, Barron WM. Enalapril and pregnancy-induced hypertension. Ann Intern Med 1988;108:911.
11. Ahokas RA, Sibai BM. The relationship between experimentally determined litter size and maternal blood pressure in spontaneously hypertensive rats. AM J OBSTET GYNECOL 1990;162:841-7.
12. Lipshitz J, Ahokas RA, Reynolds SL. The effect of hydralazine on placental perfusion in the spontaneously hypertensive rat. AM J OBSTET GYNECOL 1987;156:356-9.
13. Ahokas RA, Sibai BM, Mabie WC, Anderson GD. Nifedipine does not adversely affect uteroplacental blood flow in the hypertensive term-pregnant rat. AM J OBSTET GYNECOL 1988;159:1440-5.
14. Ahokas RA, Mabie WC, Sibai BM, Anderson GD. Labetalol does not decrease placental perfusion in the hypertensive term-pregnant rat. AM J OBSTET GYNECOL 1989; 160:480-4.
15. Koike H, Ito K, Miyamoto M, Nishino H. Effects of long-term blockade of angiotensin converting enzyme with captopril (SQ 14, 225) on hemodynamics and circulating blood volume in SHR. Hypertension 1980;2:299-303.
16. Antonaccio MJ, Rubin B, Horovitz ZP. Effects of captopril in animal models of hypertension. Clin Exp Hypertens 1980;2(3, 4):613-7.
17. Broughton Pipkin F, Wallace CF. The effect of enalapril (MK 421), an angiotensin converting enzyme inhibitor, on the conscious pregnant ewe and her fetus. Br J Pharmacol 1986;87:533-42.
18. Coen G, Cugini P, Gerlini G, Finistauri D, Cinotti A. Successful treatment of long-lasting severe hypertension with captopril during a twin pregnancy. Nephron 1985;40: 498-500.
19. Mochizuki M, Maruo T, Motoyama S. Treatment of hypertension in pregnancy by a combined drug regimen including captopril. Clin Exp Hypertens 1986;B5(1):69-78.
20. Kreft-Jais C, Plouin PF, Tchobroutsky C, Boutroy MJ. Angiotensin-converting enzyme inhibitors during pregnancy: a survey of 22 patients given captopril and 9 given enalapril. Br J Obstet Gynaecol 1988;95:420-3.

Induction of labor with pulsatile oxytocin

Karen C. Cummiskey, MD,[a] and M. Yusoff Dawood, MD[b]
Louisville, Kentucky, and Houston, Texas

In a prospective, randomized study, 106 patients underwent induction of labor with either pulsed (every 8 minutes) (n = 50) or continuous (n = 56) intravenous infusion of oxytocin. Maternal characteristics, gestational age at induction, induction-delivery interval, analgesia for labor, cesarean section rates, and newborn characteristics were similar in both groups. The mean ± SEM total oxytocin administered was significantly less in the pulsed group (3564 ± 487 mU) than in the continuous group (7684 ± 844 mU; $p < 0.0001$); the average dose of oxytocin administered per minute was significantly lower in the pulsed group (3.9 ± 0.3 mU/min) than in the continuous group (7.8 ± 0.4 mU/min; $p < 0.0001$); the peak or highest administered dose of oxytocin expressed per minute was also significantly lower in the pulsed group (9.6 ± 0.8 mU/min) than in the continuous group (14.1 ± 0.7 mU/min; $p < 0.0001$). These significant differences persisted even when controlled for parity, Bishop's score of the cervix, and number of days induction of labor was carried out. Uterine hyperstimulation occurred infrequently in both groups (3.6% to 4.0%). The pulsed group required a significantly smaller infusion volume (25 ± 14 ml) than the continuous group (780 ± 84 ml; $p < 0.0001$). Thus pulsatile administration uses significantly less oxytocin and infusion fluid but is as effective and safe as continuous infusion. (Am J Obstet Gynecol 1990;163:1868-74.)

Key words: Induction of labor, oxytocin, pulsatile oxytocin

Continuous intravenous administration of oxytocin is routinely used for induction and augmentation of labor.[1, 2] Use of oxytocin has been associated with increased maternal and fetal morbidity including uterine hyperstimulation and rupture, fetal distress with hypoxia, and an increased incidence of neonatal hyperbilirubinemia. These side effects appear to be primarily dose-dependent. With the increased utilization of intrauterine pressure monitoring and continuous fetal heart rate surveillance, these events are less common but remain potential concerns.

Endogenous oxytocin is secreted in spurts or pulses during spontaneous labor.[3] Because oxytocin binds to its myometrial receptors, causing the occupied sites to be temporarily unavailable to more continuously infused oxytocin, administration of oxytocin in pulses similar to its endogenous pulsatile secretion would appear to be a more physiologic regimen for induction of labor. Pavlou et al.[4] showed that pulsed administration of oxytocin was as effective as continuous intravenous infusion and required less oxytocin. In a recent pilot study on 20 patients, our group[5] found that pulsed administration of oxytocin for induction of labor not only was as effective as the continuous infusion but also used lower total and peak doses of oxytocin and produced a significantly lower incidence of hyperstimulation.

To further evaluate pulsatile administration of oxytocin for induction of labor, we conducted another prospective, randomized study in a larger number of patients to compare the efficacy and safety of pulsatile intravenous administration of oxytocin with the standard continuous intravenous administration of oxytocin. The patients in the pilot study reported by us[5] were not included in this analysis. This larger study would permit controlling for parity, Bishop's score, and number of days induction was performed when the two methods of giving oxytocin are compared. The results of this study are described in this paper.

Material and methods

Patient selection. One hundred six pregnant patients who required induction of labor for medical indications agreed to participate in this prospective, randomized study that was approved by the Institutional Review Board and was conducted at the University of Illinois Hospital, Chicago. Each patient gave informed consent before admission into the study and initiation of oxytocin administration. Randomization was carried out

From the Department of Obstetrics and Gynecology, University of Louisville School of Medicine,[a] and the Department of Obstetrics, Gynecology and Reproductive Sciences, The University of Texas Medical School at Houston.[b]
Supported in part by a fellowship research grant from the Illinois Section, District VI, of The American College of Obstetricians and Gynecologists.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: M. Yusoff Dawood, MD, The University of Texas Medical School at Houston, Dept. of Obstetrics, Gynecology and Reproductive Sciences, 6431 Fannin, Suite No. 3.110, Houston, TX 77030.
6/6/24723

Table I. Clinical characteristics of patients undergoing induction of labor with either pulsed or continuous oxytocin infusion

Maternal characteristic	*Pulsed oxytocin group (n = 50)*	*Continuous oxytocin group (n = 56)*	*Differences between treatment groups*
Age (yr) (mean ± SEM)	23.9 ± 0.8	23.4 ± 0.7	NS
Gravidity (mean ± SEM)	3.1 ± 0.3	2.4 ± 0.2	NS
Primigravid (No.)	21	31	
Multigravid (No.)	29	25	
Parity	1.3 ± 0.2	1.0 ± 0.2	NS
Gestational age (wk) (mean ± SEM)	39.1 ± 0.4	39.0 ± 0.3	NS
Bishop score			
Mean ± SEM	5.4 ± 0.4	5.4 ± 0.3	NS
Range	1-10	1-10	

by our pharmacy staff who assigned the patient to either group on the basis of a previously prepared randomization list. The need for induction of labor was determined by our obstetric staff. Women with a clinically contracted pelvis, previous cesarean sections, or fetal malpresentation were excluded. Fifty women were randomized to the pulsed infusion group and 56 to the continuous infusion group.

Administration of oxytocin. For pulsed administration of oxytocin, a 10-second pulse of oxytocin was delivered intravenously every 8 minutes with an Autosyringe AS-2C pump (Autosyringe, Hooksett, N.H.). The starting dose was 1 mU per pulse and the dose was doubled every 24 minutes (i.e., after three pulses of the same dose) until 32 mU per pulse. Thereafter the dose of oxytocin was increased by 8 mU per pulse until adequate uterine activity was achieved. To enable a wide range of doses available for use, the oxytocin was made up in two different concentrations of 100 or 800 mU/ml placed in a 5 ml syringe. The higher oxytocin concentration was used when a pulse dose of ≥32 mU per pulse was needed.

For continuous infusion oxytocin was given intravenously and continuously by an IMED 927 pump (Imed Corporation, San Diego). Starting with a dose of 1 mU/min, the oxytocin infusion was increased by 1 mU/min every 30 minutes until adequate uterine activity was achieved. Adequate uterine activity was defined as 160 montevideo units or more.

All cases were managed during induction of labor and delivery by our obstetric residents and faculty who also performed all the pelvic examinations. All patients had continuous electronic monitoring of fetal heart rate and uterine contractions. When uterine contractions were established, the patient underwent artificial rupture of the membranes if the cervix was dilated ≥3 cm. All monitor tracings were reviewed by one of us. Criteria for adequate labor, dysfunctional labor, and hyperstimulation were defined before the initiation of this study. Adequate (normal) labor was considered to be uterine activity demonstrating three or more uterine contractions in a 10-minute period with 160 montevideo units or more. Dysfunctional labor patterns were defined as coupling or tripling of uterine contractions for a total of ≥1 hour during induction of labor. Hyperstimulation was present if five or more uterine contractions were noted in succession without return to baseline tonus or a prolonged uterine contraction causing fetal bradycardia. Either of these two conditions required temporary cessation of oxytocin administration.

Statistical analysis. The means ± SEMs between the pulsed and continuous oxytocin infusion groups were analyzed for differences by means of χ^2 analysis, nonpaired Student's t test, and one-way analysis of variance. Two-tailed p values were used, and values >0.05 were considered not significant.

Results

The indications for induction of labor were similar in both groups. These included postdates, hypertension, preeclampsia, diabetes, intrauterine growth retardation, and premature rupture of membranes. In the pulsed group 30 patients required 1 day of attempted induction, 16 required 2 days, and 4 required 3 days. In the continuous group 35 women were delivered after 1 day of induction, 12 after 2 days, and 9 after 3 days.

The clinical characteristics of the patients are summarized in Table I. There were no significant differences with regard to maternal age, gravidity, gestational age, or Bishop scores at the time of induction. Table II summarizes the events during labor. The induction-to-delivery time, as reflected by the length of time the patient received oxytocin, was similar in the two groups. Use of pain medications and epidural anesthesia was not significantly different. Both groups had a low incidence of uterine hyperstimulation—4% in the pulsed group and 3.6% in the continuous group. Nevertheless, the pulsed group used a significantly smaller volume of fluid (25 ± 14 ml) than the continuous group (780 ± 84 ml) to deliver the oxytocin ($p < 0.0001$).

Table II. Labor characteristic of patients undergoing induction of labor with either pulsed or continuous oxytocin infusion

Labor characteristic	*Pulsed oxytocin group (n = 50)*	*Continuous oxytocin group (n = 50)*	*Difference between treatment groups*
Total time oxytocin given (min) (mean ± SEM)	846 ± 68	930 ± 74	NS
Total fluid received with infusion (ml) (mean ± SEM)	25 ± 13	780 ± 85	$p < 0.0001$
Pain medication (%)	52	41	NS
Epidural anesthesia (%)	14	29	NS
Contraction pattern (%)			
Normal	88	87.5	NS
Dysfunctional	8	8.9	NS
Hyperstimulation	4	3.6	NS

Table III. Characteristics of newborns delivered of patients undergoing induction of labor with either pulsed or continuous oxytocin infusion

Newborn characteristic	*Pulsed oxytocin group (n = 50)*	*Continuous oxytocin group (n = 56)*	*Difference between treatment groups*
Birth weight (gm) (mean ± SEM)	3185 ± 98	3255 ± 93	NS
Apgar score at 1 min			
Mean ± SEM	7.7 ± 0.2	7.8 ± 0.1	NS
Range	3-9	5-9	
<7 (No.)	6	6	
APGAR score at 5 min			
Mean ± SEM	8.6 ± 0.1	8.8 ± 0.1	NS
Range	5-9	7-10	
<7 (No.)	1	0	
Arterial cord blood pH (mean ± SEM)	7.30 ± 0.02	7.29 ± 0.02	NS
	(n = 14*)	(n = 14)	
Hyperbilirubinemia (No.)	3	5	NS
	(n = 26)	(n = 31)	

*Numbers studied for arterial cord pH and for neonatal serum bilirubin are shown in parentheses.

Forty-seven women (94%) in the pulsed group were delivered vaginally, with 3 (6%) requiring cesarean section, while 7 of the 49 (12.5%) in the continuous group were delivered by cesarean section. The indications for cesarean section were similar in both groups.

Relevant findings of the newborns are summarized in Table III. There were no differences between the two groups with regard to birth weight or Apgar scores at 1 and 5 minutes. Arterial cord pH determined in 14 patients from each group showed no significant differences. Of those infants screened independently by the pediatricians for hyperbilirubinemia in the newborn period, three infants were identified in the pulsed group and five infants in the continuous group. Although these numbers are not significantly different, when analyzed further, all three infants with hyperbilirubinemia in the pulsed group had a mean gestational age of 35 ± 1.0 weeks and a mean birth weight of 1953 ± 116 gm. The maximum serum bilirubin level was 11.7 ± 0.5 mg/dl. In contrast, the hyperbilirubinemic infants in the continuous group had a mean gestational age of 37.4 ± 1.2 weeks with a significantly higher mean birth weight of 3830 ± 184 gm ($p < 0.01$) and a significantly higher maximum serum bilirubin of 15.1 ± 0.7 mg/dl ($p < 0.001$).

Fig. 1 shows the mean ± SEM total dose of oxytocin administered during induction of labor for each group. The pulsed group used significantly less oxytocin with a mean total dose of 3564 ± 487 mU while the continuous group used 7684 ± 844 mU ($p < 0.0001$). To control for the number of days required for induction, the total dose of oxytocin was further evaluated according to the number of days labor was induced, as shown in Fig. 2. The pulsed group continued to demonstrate a significantly lower total oxytocin requirement than the continuous group regardless of the number of days of induction. To determine and control for the possible influence of the Bishop score of the cervix on

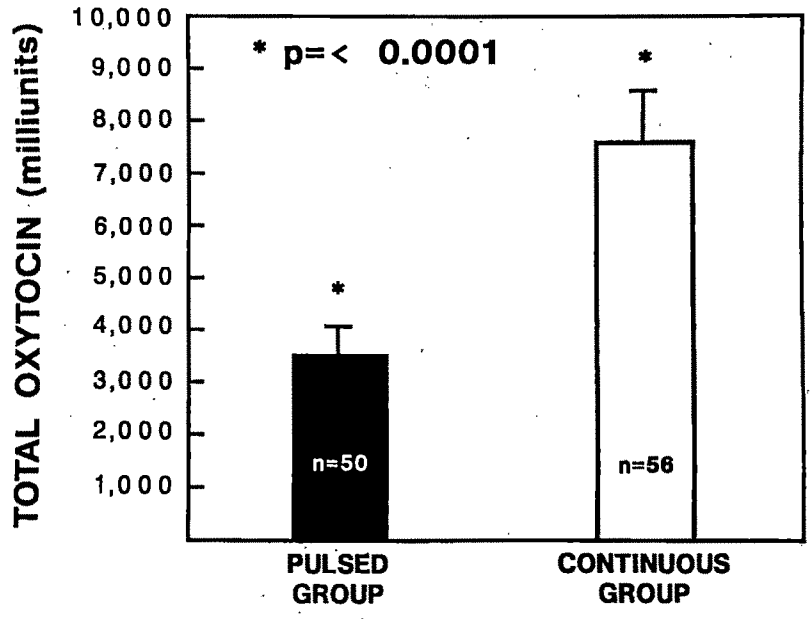


Fig. 1. Total dose of oxytocin (mean ± SEM) in patients receiving pulsed oxytocin compared with continuous oxytocin for induction of labor. The pulsed group received a significantly lower mean total dose of oxytocin as compared with the continuous group.

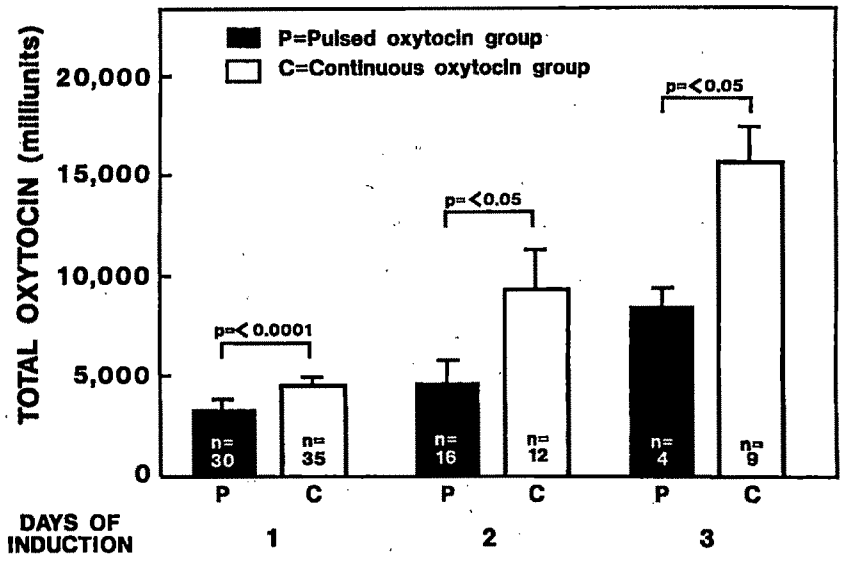


Fig. 2. Total dose of oxytocin (mean ± SEM) in patients receiving pulsed oxytocin compared with continuous oxytocin grouped according to the number of days of attempted induction of labor. The pulsed group always required significantly less oxytocin than the continuous group.

the total dose of oxytocin required for induction, the total dose of oxytocin required was reanalyzed for patients with low (0 to 4), moderate (5 to 8), and high (9 to 13) Bishop scores (Fig. 3). Within each of these three categories, the pulsed group consistently required significantly less total oxytocin than the continuous group ($p < 0.5$).

When the mean ± SEM doses of oxytocin in milliunits per minute for the two methods of giving oxytocin were compared, the pulsed group again had a significantly lower mean dose (3.9 ± 0.3 mU/min) than the continuous group (7.8 ± 0.4 mU/min; $p < 0.0001$). The mean dose of oxytocin per minute continued to be significantly lower with pulsed administration than with continuous administration when controlled for the number of days of induction and

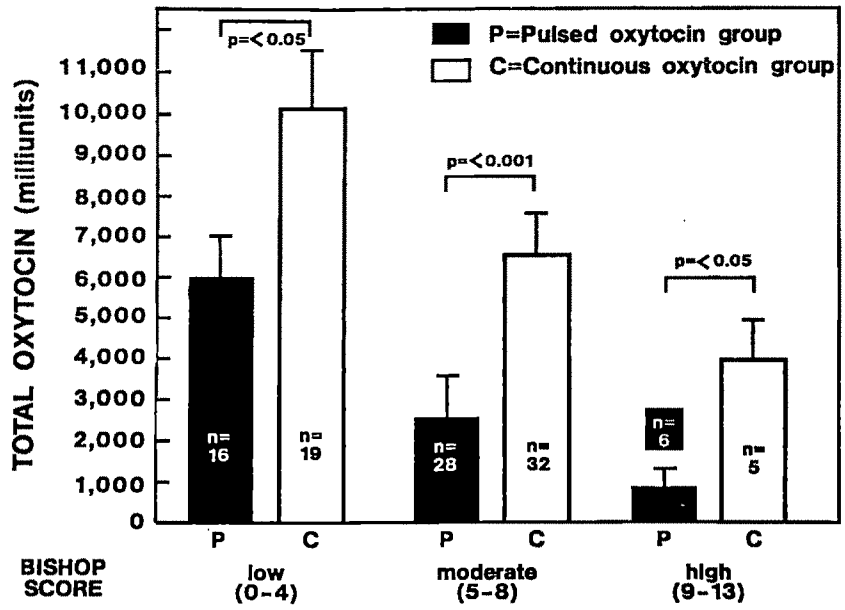


Fig. 3. Total dose of oxytocin (mean ± SEM) in patients receiving pulsed oxytocin compared with continuous oxytocin grouped according to the Bishop cervical scores of the patients. Irrespective of group according to Bishop scores, significantly less oxytocin was always used for pulsed administration than for continuous administration. Note that there was a more dramatic progressive drop in the total dose of oxytocin with pulsed administration as the Bishop scores progressed from low to moderate to high.

for low, moderate, and high Bishop scores (data not shown).

The highest or peak dose of oxytocin amortized per minute was also evaluated. The pulsed group had a lower peak dose of oxytocin (9.5 ± 0.8 mU/min) when compared with the continuous group (14.1 ± 0.7 mU/min; $p < 0.0001$). Again, the difference remained significant when controlled for the number of days of induction and for Bishop scores.

To remove the potential confounding influence that patients requiring cesarean section may have on those who are delivered vaginally with each method of administering oxytocin, the data on oxytocin for each group were reanalyzed only for those patients who achieved vaginal delivery but are not shown in the figures. The total dose of oxytocin remained significantly lower for the pulsed group (3218 ± 408 mU; $n = 47$) than for the continuous group (6985 ± 778 mU; $n = 49$; $p < 0.0001$). Likewise, the mean dose and the peak dose of oxytocin were significantly lower in the pulsed group (3.7 ± 0.3 and 9.3 ± 0.8 mU/min, respectively) than in the continuous group (7.6 ± 0.4 and 13.8 ± 0.8 mU/min, respectively; $p < 0.0001$ for both).

Comment

Our results demonstrate that pulsatile administration of oxytocin is as safe and as effective as continuous oxytocin infusion for induction of labor. Previous studies on induction of labor in smaller numbers of patients and on augmentation of labor found significantly less oxytocin was required when given by pulsatile administration than by continuous administration.[1-6] This study confirmed those previous reports demonstrating a lower total dose and mean and peak doses (expressed per minute) of oxytocin for induction of labor with pulsed oxytocin administration. The significant reduction in the dose of oxytocin required to induce labor with pulsed oxytocin persisted even when controlled for the number of days of attempted induction, the Bishop scores of the cervix, and the number of cesarean sections. Although we have recently also found that less oxytocin was requird to *augment* labor, the oxytocin needs for induction of labor are not similar to the requirements for augmentation. Because the number of patients in the present induction study is larger than in our preliminary study,[5] we are able to demonstrate a clear correlation between the total amount of oxytocin required and cervical ripeness (as reflected by the Bishop score) with both methods of oxytocin administration. The inverse relationship between the total dose of oxytocin required and the Bishop score was more acute with pulsed administration than with continuous administration of oxytocin.

Several basic and practical considerations predicated

our choice of the pulse frequency for administering pulsatile oxytocin and for the interval between dose increments. Endogenous oxytocin is released in frequent pulses or spurts during pregnancy and labor in women.[3, 7] Dawood et al.[3] have shown that during normal spontaneous labor, maternal plasma oxytocin levels show two to three pulses every 10 minutes.[8] Pharmacokinetic studies of oxytocin gave a half-life of 10 minutes and a metabolic clearance rate that was similar in both pregnant and nonpregnant subjects.[9] On the basis of the pulse frequency and pharmacokinetics of oxytocin and for convenience of adjusting the Autosyringe pump, we chose a pulse frequency of every 8 minutes for administration of the oxytocin. Seitchik and Castillo[10] have emphasized that the dose increment and the rate at which the dose is increased are critical determinants of both the maximum dose and the frequency of hypercontractility and/or fetal distress. Previous studies of constant intravenous infusion of oxytocin found that the minimum time required to reach a steady-state circulating level of oxytocin ranged from 20 to 40 minutes.[8, 11] An intermediate interval of 30 minutes was used before the dose of oxytocin was increased stepwise.

The action of oxytocin on the uterus is brought about through specific receptors that have been identified in the cell membranes of the smooth muscles of the uterus. The number of oxytocin receptors increases as gestation progresses. When these receptors are bound by oxytocin, they are no longer available, albeit temporarily, to more circulating oxytocin. Whereas the duration of oxytocin-receptor occupancy by oxytocin is not known, continuous infusion of oxytocin can be expected to rapidly saturate the uterine oxytocin receptors, thus giving rise to more circulating oxytocin than can be used by the uterus. Pulsed administration of oxytocin, which is similar to its endogenous release, is therefore a more physiologic method for giving oxytocin to stimulate uterine contractions.

The total dose of oxytocin required to establish contractions and maintain labor was significantly less with pulsed administration than with the continuous infusion. Because most complications of oxytocin are dose-related, reduction in the total dose will allow an even wider margin of safety. In a preliminary study we have previously demonstrated a significantly lower incidence of hyperstimulation with the pulsed administration of oxytocin for induction of labor.[5] In this study, involving a much larger number of patients, the incidence of hyperstimulation was low in both groups.

The risk of neonatal hyperbilirubinemia is dependent on both the total dose of oxytocin given to the mother and the maturity of the fetus.[12-14] In in vitro experiments, oxytocin increases osmotic swelling and deformability of erythrocytes in a time- and dose-dependent manner to bring about hemolysis, an effect similar to that of vasopressin.[15] An increased osmotic fragility of the erythrocytes associated with transplacental hyponatremia and its accompanying hypoosmolality has been found in neonatal hyperbilirubinemia related to oxytocin given to the mother.[14, 16] Singhi and Singh[15, 16] concluded that the transplacental passage of oxytocin administered to the mother could be responsible for the significant lowering of the cord sodium level and osmolality found with an oxytocin infusion >16 mU/min compared with a lower dose. Buchan[14] found an increased incidence of neonatal hyperbilirubinemia when mothers were given a mean total oxytocin dose of ≥4500 mU. Therefore pulsed administration of oxytocin with a mean total dose of 3200 to 3600 mU as found in this study should afford a wider margin of safety, especially for risk of oxytocin-associated neonatal hyperbilirubinemia, when compared with the higher mean total dose of 7000 to 7700 mU in the continuous infusion group. Three infants in the pulsed oxytocin group and five in the continuous oxytocin group developed clinically recognizable and biochemically confirmed neonatal hyperbilirubinemia. While these numbers are small, it is particularly noteworthy that all three infants with hyperbilirubinemia in the pulsed oxytocin group were preterm with low birth weights and comparatively lower maximum levels of serum bilirubin before therapy. None of the term infants in the pulsed group had hyperbilirubinemia. In contrast, the five infants in the continuous infusion group were more advanced in gestation, were heavier in birth weight, and had higher maximum levels of serum bilirubin. In addition to the dose of oxytocin, the maturity and size of the fetus are important variables in the risk of development of neonatal hyperbilirubinemia; therefore a larger number of patients will have to be studied so that the variability in weight and maturity of the newborn can be adequately controlled while the effect of the total dose of oxytocin on the risk of neonatal hyperbilirubinemia is examined.

Requiring a significantly smaller amount of infusion fluid to administer the oxytocin, pulsed administration may be advantageous in high-risk conditions requiring close monitoring or restriction of fluids such as in women with severe preeclampsia, pulmonary edema, or cardiac and renal diseases.

In conclusion, we found that pulsed administration of oxytocin is as safe and effective as continuous infusion, requires significantly less oxytocin with a wider margin of safety, and may be a more physiologic oxytocin regimen for induction and augmentation of labor. Further studies are indicated to determine the optimal pulse intervals and rates of increase of oxytocin when

Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist

Laird Wilson, Jr., PhD,[a] Michael T. Parsons, MD,[b] and George Flouret, PhD[c]
Chicago, Illinois, and Tampa, Florida

The effect of a potent oxytocin antagonist, produced in our laboratories, on spontaneous uterine contractions in the pregnant baboon was examined. Three types of uterine contractions were studied: immediately after operation, during the nocturnal period, and near or at labor. Bolus intravenous injections of oxytocin antagonist were given and uterine activity was examined ±1 hour after the injection. The oxytocin antagonist caused a precipitate decrease (approximately 70%) in contractile force (mean amplitude × frequency) in the first 15 minutes after injection ($p < 0.05$); this force diminished to approximately 90% at the end of 1 hour for both nocturnal and labor contractions. In contrast, uterine contractions immediately after operation were diminished by only 60% within 60 minutes after the oxytocin antagonist. These results indicate that the oxytocin antagonist is a potent inhibitor and suggest that oxytocin is a primary regulator of spontaneous nocturnal and labor uterine contractions in the pregnant baboon. (AM J OBSTET GYNECOL 1990;163:1875-82.)

Key words: Oxytocin antagonist, pregnancy, tocolytic, preterm labor, baboon

The mechanism of parturition in the primate is poorly understood. A number of factors have been implicated in either the initiation or the progress of labor, including oxytocin,[1] prostaglandins,[2] leukotrienes,[3] and the ratio of estrogen to progesterone.[4] The role of oxytocin in initiation of labor is ambiguous because of the contradictory reports in the literature as to the correlation of oxytocin levels in the plasma of humans and nonhuman primates with advancing gestational age and labor.[5-8] The clinical importance of understanding the initiation and progress of labor is to apply this knowledge to control preterm labor, a major cause of fetal mortality and morbidity in obstetric medicine.

We have recently reported the development of a potent oxytocin antagonist that inhibits in vitro uterine contractions in response to oxytocin in tissue from rats, baboons, and humans,[9] inhibits in vivo contractions in rats[9] and baboons in response to exogenous oxytocin, and delays the progress of labor in rats.[9] We report here the ability of the oxytocin antagonist to inhibit spontaneous uterine contractions during the last trimester of pregnancy in the baboon. Our results show that the oxytocin antagonist is a potent inhibitor of nocturnal uterine contractions and contractions related to labor and suggest that oxytocin is a primary regulator of these types of uterine contractions during pregnancy. The data imply that the oxytocin antagonist has the potential of inhibiting uterine contractions of preterm labor in humans.

From the Department of Obstetrics and Gynecology, University of Illinois at Chicago, College of Medicine,[a] the Department of Obstetrics and Gynecology, University of South Florida, College of Medicine,[b] and the Department of Physiology, Northwestern University Medical School.[c]
Supported in whole or in part by National Institutes of Health grant No. HD 22567 and The Earl M. Bane Charitable Trust.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, March 15-18, 1989, San Diego, California.
Reprint requests: Laird Wilson, Jr., PhD, Department of Obstetrics and Gynecology, University of Illinois at Chicago, College of Medicine, 840 South Wood St., Chicago, IL 60612.
6/6/24767

Material and methods

Baboons *(Papio anubis)* with timed pregnancies from the breeding colony of the Biological Resource Laboratory at the University of Illinois were used in these studies. The animals were kept under controlled lighting conditions (12 hours light/12 hours dark) with lights on at 6 AM (Central Standard Time) and fed Purina Primate Chow (Ralston Purina, St. Louis) fruit, and nuts once a day and water as desired. Normal delivery occurs at 184 ± 2 days. The protocol for this report has been approved by the Animal Care Committee at the University of Illinois.

Surgical procedures and postsurgery care. All baboons were operated on between 125 and 135 days of pregnancy. Before operation, ultrasonography was performed on all animals to determine fetal viability, as well as the location of the placenta and pockets of amniotic fluid for inserting the cannulas. Animals were initially sedated with an intramuscular injection of ketamine (7 mg/kg) and an intravenous injection of Surital (Parke Davis, Division of Warner-Lambert Co., Morris Plains, N.J.) (2 ml intravenously of a 2.5% solution). Anesthesia was maintained with 0.8% to 1.0% halothane (Wyeth-Ayerst, Philadelphia) during the operation. A midline incision was made just above and

lateral to the umbilicus. Two 0.86 × 1.32 mm vinyl cannulas (Bolabs Inc., Lake Havasu City, Ariz.) were inserted within 4 cm of each other directly through the uterine wall into the amniotic fluid via a 16-gauge needle acting as a trocar. Location of the cannula insertion was determined by the previous ultrasonogram. Approximately 15 inches of cannula was threaded into the amniotic fluid. The cannulas were then anchored to the serosal layer of the uterus with a 3-0 silk suture. The cannulas were exteriorized via a midlateral flank incision with a subcutaneous tunneling needle (Alice King Chatham Medical Art, Los Angeles). The midventral incision was closed with Vicryl suture. An incision was made in the groin area of the leg just above the external femoral vessels and artery. The vein and artery were dissected free and 0.86 × 1.32 mm vinyl tubing was placed into incisions in the vein and artery. A sufficient length of tubing (approximately 10 inches) was placed into the vein so that the tip rested in the vena cava or aorta just above the bifurcation, as determined by the location of the abdominal umbilicus. The cannulas were anchored with 3-0 silk suture and were exited subcutaneously at the junction of the leg and abdomen with tunneling needles. The leg incision was closed with 2-0 Vicryl (Ethicon, a subsidiary of Johnson and Johnson, Somerville, N.J.) suture. The four cannulas (two amniotic fluid, one femoral artery, and one femoral vein) were led subcutaneously to a middorsal, area just inferior to the scapula. The skin incisons for tunneling were then closed with 2-0 Vicryl. A nylon mesh jacket (Alice King Chatham Medical Art) was placed on the baboon, and the cannulas were led out through a flexible stainless steel tether attached to the back of the jacket. The length of the tether was 24 inches. The tether was attached to a swivel on the side of the cage; this training swivel had been punched out in the middle. This technique allowed the cannulas to be led directly out of the cage and attached to pressure transducers or infusion pumps.[3] The amniotic fluid cannulas were attached to Gould model P23id pressure transducers and pressure changes were recorded on a four-channel, model 7 Grass polygraph (Grass Instruments, Quincy, Mass.). In addition, the analog signal of the intrauterine pressure changes was digitized with a Data Translation 2811 H board on a Dell PC Limited 286 computer, and these data were stored on a hard disk. This signal was sampled every second.

The cannulas in the femoral vein and artery were attached to syringes on a Harvard infusion pump (Harvard Apparatus Co., Millis, Mass.). Heparin (100 U/ml) and Kefzol antibiotic (Eli Lilly & Co., Indianapolis), 71.4 mg/ml in saline solution, were infused at a rate of 0.582 ml/hr to keep the cannulas patent and deliver 1 gm of Kefzol antibiotic per day to the animals. After the first week the Kefzol was reduced to 0.5 gm/day. We have had few maternal infections using this method.

Oxytocin antagonist. The solid phase synthesis of the oxytocin antagonist [β-mercapto-β,β-cyclopentamethylene propionic acid-D-Trp^2-Phe^3-Ile^4-Arg^8]-oxytocin has been previously described.[10]

Uterine contractility studies. Uterine contractions were monitored 24 hours a day through delivery. The oxytocin antagonist was tested on three types of uterine contractions: (1) immediately after operation, (2) during nocturnal activity, and (3) near (≤3 days) or at labor. The oxytocin antagonist was given as an intravenous bolus injection in the range of 500 to 3000 nmol, except in preliminary studies where the oxytocin antagonist was infused for various lengths of time (e.g., Fig. 1). The uterine contractile activity was examined 1 hour before and after the oxytocin antagonist was administered for mean amplitude, frequency, contractile force (mean amplitude × frequency), and percent contractile force per 15-minute interval. The percent contractile force was determined by dividing all values by the mean of the pre–oxytocin antagonist administration values. We examined the effect of the oxytocin antagonist on uterine activity of seven animals immediately after operation and compared it with uterine activity in six controls. Operation was usually completed by 2 to 3 PM, and the oxytocin antagonist was given between 6 and 8 PM that same evening. The measurements for the controls were performed at the same time of evening as the oxytocin antagonist–treated animals. Studies examining nocturnal uterine contractions involved 10 animals. The controls chosen for the nocturnal uterine contractions were those contractions that occurred within ±2 days of those in the treated animals at the same time of evening. Since uterine activity was not always detectable with these criteria, there were fewer control (N = 19) than treated (N = 47) studies.

Animals having labor-like contractions were defined as having a contractile force >120 per 15-minute interval occurring within 3 days of delivery. The contractile force of controls for animals in labor was determined from uterine activity occurring within 2 hours of delivery, but before abdominal pushing occurred, and meeting the criteria of a contractile force of 120 per 15-minute interval for labor-like contractions. There were four oxytocin antagonist–treated and five control animals in this study.

Statistical analysis. The data were analyzed by grouping variables (Treatment – Control vs oxytocin antagonist treated) with within-repeat-measures (Time)

analysis of variance, and when significant, differences between means were detected by Tukey's test.[11]

Results

The effect of infusing the oxytocin antagonist into a pregnant baboon showing significant uterine contractile activity is shown in Fig. 1. The uterine activity is expressed as contractile force (mean amplitude × freuqency per ½-hour intervals) during the days illustrated. As determined from preliminary studies, uterine activity was considered significant and labor-like when the contractile force was >240 per 30 minutes (e.g., 12 contractions × 20 mm Hg = 240). When the oxytocin antagonist was administered on day 154 as an intravenous bolus injection of 3000 nmol followed by infusion at 16 nmol/min for 1½ days, uterine activity was immediately suppressed and remained at baseline for the period of the infusion (Fig. 1, *A*). Ten days later (day 164) uterine activity again increased significantly, but this time the oxytocin antagonist was infused for 2 hours and induced a precipitate decrease in uterine contractions. The following day (day 165) uterine activity again increased, and the oxytocin antagonist was infused for 1 hour, resulting in immediate suppression of uterine activity (Fig. 1, *B*). Fig. 1, *C*, shows the contractile force when the animal was allowed to deliver. This, plus other data, suggested that the oxytocin antagonist did not have to be continuously infused to be effective but rather that a bolus injection might suffice. Thus for all subsequent studies a bolus injection of oxytocin antagonist was given.

Fig. 2 illustrates a computer-generated, digitized, analog signal of uterine activity in a baboon showing strong uterine activity and about to deliver. At day 176 of pregnancy (Fig. 2) 3000 nmol of oxytocin antagonist was given as an intravenous bolus injection. Contractile activity was immediately suppressed and remained at baseline for the remainder of the evening. Because of the strength of these contractions and the uterine activity during the preceding nights, it is likely that, if not suppressed, delivery would have occurred. The next day this animal went into labor as determined by the fact that the membranes ruptured, and mucus and blood were on the cage floor and in the vaginal area. A bolus injection of 3000 nmol of oxytocin antagonist caused a precipitate drop in uterine activity, but it was not toally eliminated. To determine if the uterine contractile activity could be totally suppressed, a second bolus injection of oxytocin antagonist was given about 2½ hours after the first injection, followed 1½ hours later with a continuous infusion of oxytocin antagonist at 16 nmol/min for 3 hours. The combination of the two bolus injections of oxytocin antagonist and the infusion totally eliminated uterine activity. The computer compression of the data (Fig. 3) gives the appearance of some activity, represented as spikes on the chart, but when the data were examined at normal spacing, the

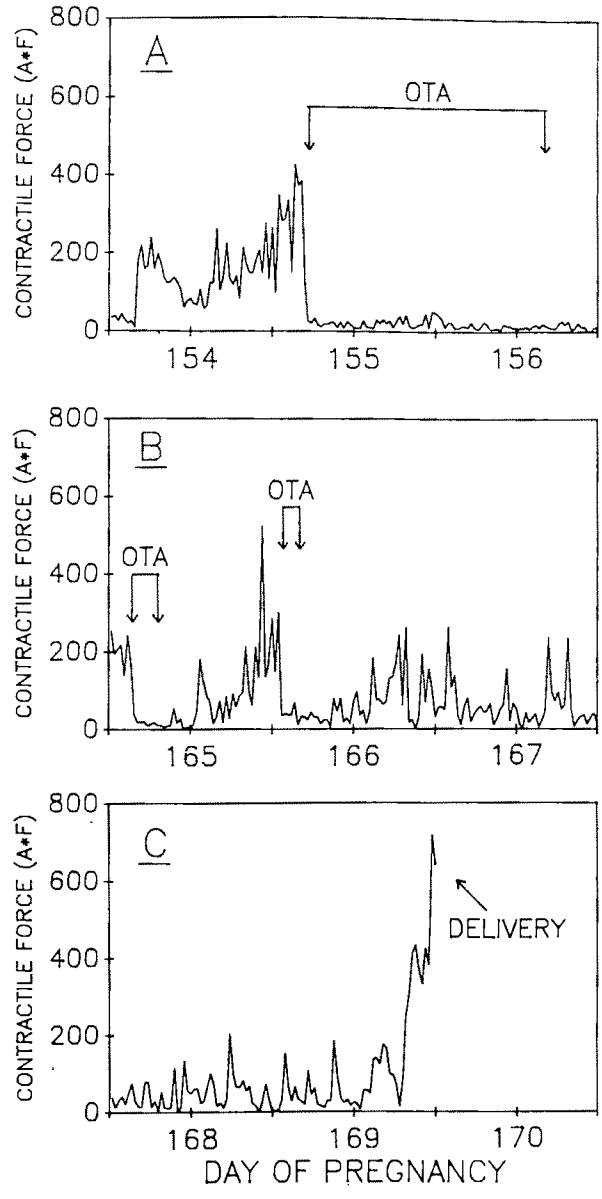


Fig. 1. This figure illustrates the effect of infusing the oxytocin antagonist *(OTA)* in an individual baboon at three different days of pregnancy when the contractile force (mean amplitude × frequency) per 30-minute interval reaches significant levels. At day 154 **(A)** the oxytocin antagonist was given initially as a bolus injection of 3000 nmol plus infused at 16 nmol/min for 1½ days. The oxytocin antagonist caused a precipitate drop in contractile force, and this force stayed at baseline during the infusion period. Similar results were noticed **(B)** when the oxytocin antagonist was infused for 2 hours (day 164) and 1 hour (day 165). **C** shows the changes in contractile force that occurred when the animal was allowed to deliver.

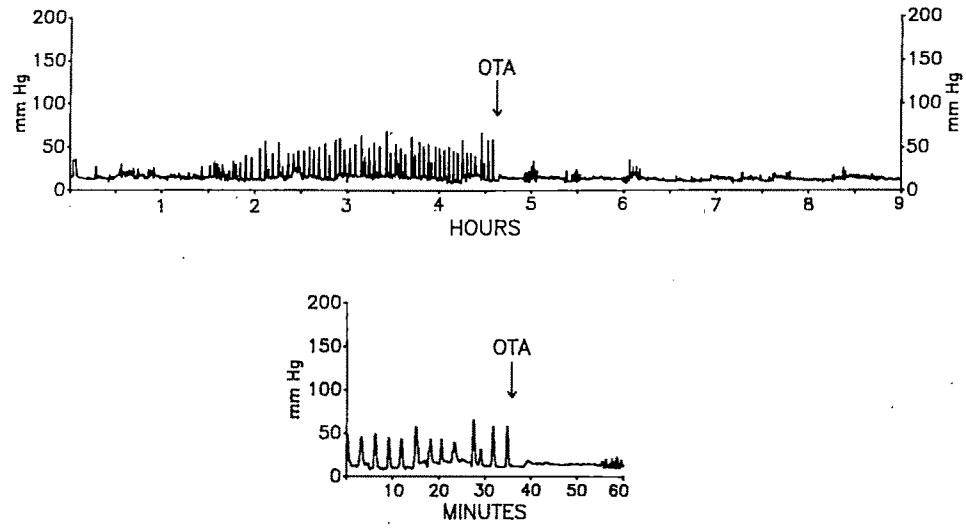


Fig. 2. Illustrated in the *top chart* is the computer-generated, digitized signal of uterine activity in a baboon showing strong nocturnal activity. As soon as an intravenous bolus injection of 3000 nmol of the oxytocin antagonist was given, contractile activity diminished and stayed at baseline the remainder of the evening. *Bottom,* An expanded view of uterine activity at the time the oxytocin antagonist was administered.

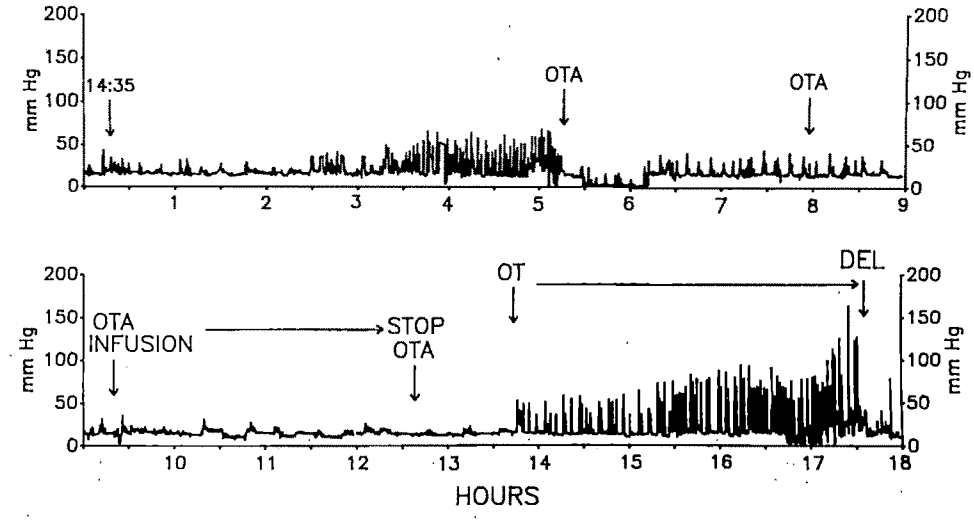


Fig. 3. Depicted in this figure is the same baboon as in Fig. 2 except labor had begun. We assumed delivery was imminent because the membranes had ruptured, mucus and blood were on the cage floor and in the vaginal area, and she was showing abdominal pushing. Bolus injections of the oxytocin antagonist (3000 nmol) caused a precipitate drop in uterine activity, but it was not totally eliminated. However, when the oxytocin antagonist was infused at 16 nmol/min, uterine contractile activity was totally suppressed. One hour after the oxytocin antagonist infusion was stopped, oxytocin *(OT)* was infused and induced immediate contractile activity that led to delivery of a live fetus in about 3½ hours.

baseline was essentially flat. After oxytocin antagonist was stopped 1 hour, the animal showed no uterine activity. To determine if the uterus would respond to exogenous oxytocin after oxytocin antagonist was stopped, oxytocin was infused starting at 10 nmol/min (5 mU/min) and stepwise increased to 50 nmol/min. Uterine contractions started immediately on oxytocin infusion; a live fetus was delivered 3½ hours later.

Figs. 4 and 5 depict the summary of all data related to nocturnal uterine activity examined within ± 1 hour at 15-minute intervals before or after oxytocin antagonist administration and expressed as amplitude, frequency, contractile force, and percent contractile force. Amplitude, frequency (Fig. 4, *A* and *B*), and contractile force (Fig. 5, *A*) showed significant decreases ($p < 0.05$) within the first 15-minute interval after the oxytocin

antagonist administration, with contractile force reflecting the most dramatic change. The control values before oxytocin antagonist (−60, −45, −30, and −15 minutes) were significantly ($p < 0.05$) less than the corresponding oxytocin antagonist group, reflecting the rationale for administering the oxytocin antagonist, which was to suppress significant uterine contractions that might lead to preterm labor. When expressed as a percentage of the baseline, the contractile force was inhibited by 70% ($p < 0.05$) within 15 minutes, and this force diminished to 90% after 1 hour (Fig. 5, *B*). The control group did not show a significant ($p > 0.05$) decrease in uterine activity over the same time intervals. The mean dose of oxytocin antagonist ± SE for 47 intravenous bolus injections was 1921.3 ± 137.5 nmol with a range of 500 to 3000 nmol. Of the 47 injections of oxytocin antagonist administered, 40 (i.e., 85%) resulted in significant suppression of uterine activity for the remainder of the evening.

When animals were near or at labor, the mean contractile force ± SEM for the oxytocin antagonist–treated group at −60, −45, −30, −15, +15, +30, +45, and +60 minutes was: 151.0 ± 20.6, 162.0 ± 26.7, 127.6 ± 15.5, 152.3 ± 33.1, 25.1 ± 7.6, 14.0 ± 5.2, 13.3 ± 3.8, and 11.4 ± 4.2, respectively. Similarly, the control values were: 209.0 ± 54.1, 130.0 ± 36.2, 164.0 ± 28.3, 138.0 ± 29.2, 128.5 ± 20.3, 187.5 ± 32.1, 160.0 ± 28.5, and 243.0 ± 34.9, respectively. The mean dose of oxytocin antagonist administered ± SEM for 10 intravenous bolus injections was 2500.0 ± 223.6 nmol with a range of 1000 to 3000 nmol. Animals received the oxytocin antagonist during the last 2 to 3 days before and/or during delivery. These results are summarized as percent contractile force in Fig.6. As with nocturnal activity, the oxytocin antagonist caused an immediate suppression of the percent contractile force ($p < 0.05$). In addition, one of the animals also received the oxytocin antagonist during the second stage of labor (defined as abdominal pushing in the intrauterine pressure recordings) and the uterine activity was immediately suppressed.

The mean contractile force ± SEM for animals immediately after operation for 60 minutes before or after the oxytocin antagonist administration at the same time interval listed above for nocturnal activity was: 143.3 ± 22.0, 126.7 ± 29.1, 119.0 ± 24.9, 145.8 ± 28.8, 55.4 ± 10.3, 40.5 ± 6.7, 39.2 ± 10.8, and 54.3 ± 10.0, respectively. Similarly, the results for the controls were: 73.0 ± 10.3, 60.1 ± 11.3, 72.6 ± 21.9, 55.0 ± 14.6, 36.1 ± 8.9, 62.0 ± 15.3, 55.5 ± 13.7, and 49.5 ± 8.9, respectively. When uterine activity was expressed as percent contractile force and compared with nocturnal and labor contractions (Fig. 6), the oxytocin antagonist caused a significant decrease ($p < 0.05$) related to operation, but activity was usually not suppressed to baseline as often occurred with nocturnal and labor

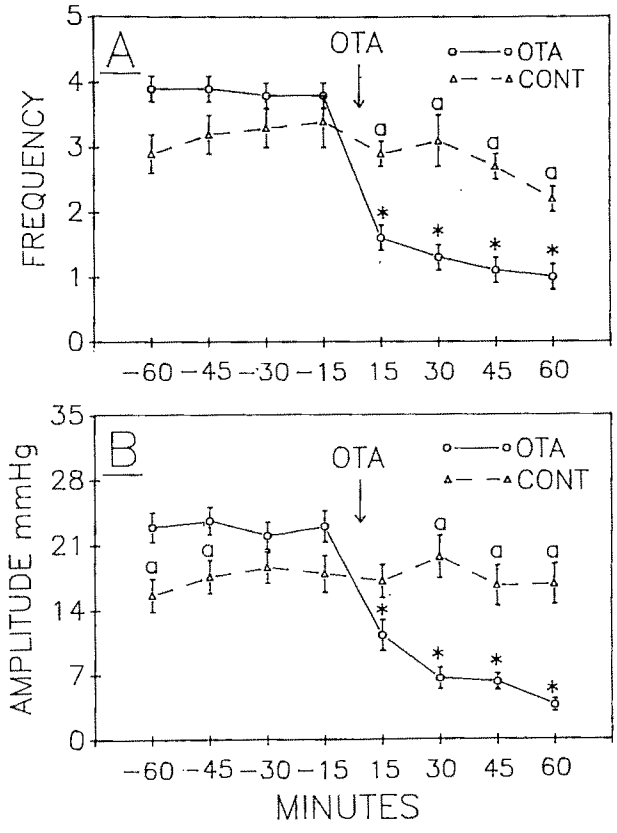


Fig. 4. This figure summarizes the effects of the oxytocin antagonist on the frequency **(A)** and amplitude **(B)** of nocturnal uterine activity 60 minutes before or after an intravenous bolus injection of the oxytocin antagonist was administered. The oxytocin antagonist caused a decrease (*asterisk*, $p < 0.05$) in frequency and amplitude within the first 15 minutes after injection of the oxytocin antagonist when compared with preinjection levels. No significant changes ($p > 0.05$) were seen for the controls when compared with their own baseline. However, when control and treated values were compared, differences were detected (*a*, $p < 0.05$). $N = 47$ and $N = 19$ for oxytocin antagonist and controls, respectively. *OTA*, Oxytocin antagonist. *CONT*, Controls. Values are mean ± SEM.

contractions. At the end of 60 minutes the percent contractile force was about 40% of baseline compared with 10% for nocturnal and labor contractions, and this was significantly different ($p < 0.05$). The mean dose of oxytocin antagonist administered ± SEM was 2416.7 ± 271.3 nmol for six intravenous bolus injections with a range of 1500 to 3000 nmol.

Comment

The results of this study show that an oxytocin antagonist is an effective inhibitor of spontaneous uterine contractions related to nocturnal activity and labor in the baboon. These data have several important implications. First, they suggest that oxytocin is a primary regulator of uterine activity during pregnancy. This does not mean that other factors (e.g., estradiol and

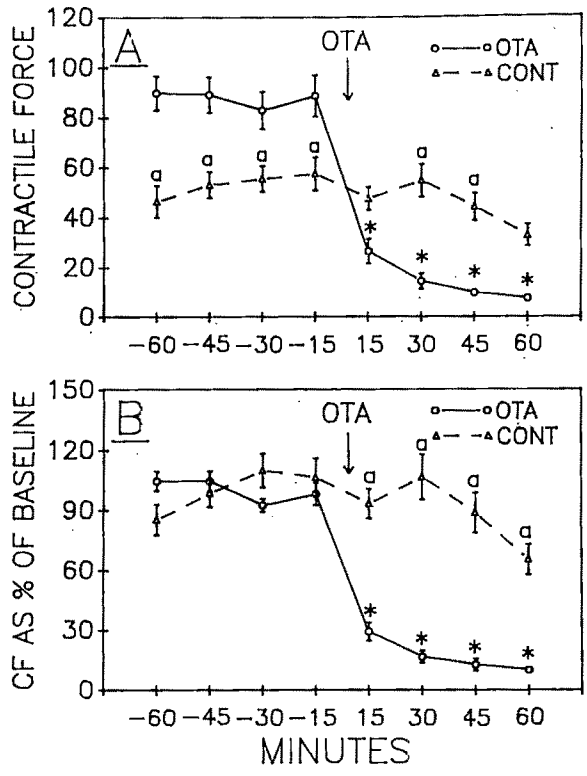


Fig. 5. The same nocturnal uterine activity data shown in Fig. 4 are shown, but they are expressed as contractile force **(A)** and percent contractile force **(B)**. A bolus injection of the oxytocin antagonist caused a precipitate drop (*asterisk*, $p < 0.05$) in uterine activity of about 70% within 15 minutes, and this activity diminished further to 90% inhibition within 60 minutes after the oxytocin antagonist injection when compared with baseline. No differences ($p > 0.05$) were found in controls when compared with baseline values, but differences were detected (*a*, $p < 0.05$) when compared with treated animals. The percent contractile force in **B** was determined by averaging the preinjection oxytocin antagonist data and dividing all values by this mean. *OTA*, Oxytocin antagonist. *CONT*, Controls. Values are mean ± SEM. Contractile force = (mean amplitude × frequency)/15 minutes. $N = 47$ and $N = 19$ for oxytocin antagonist and controls, respectively.

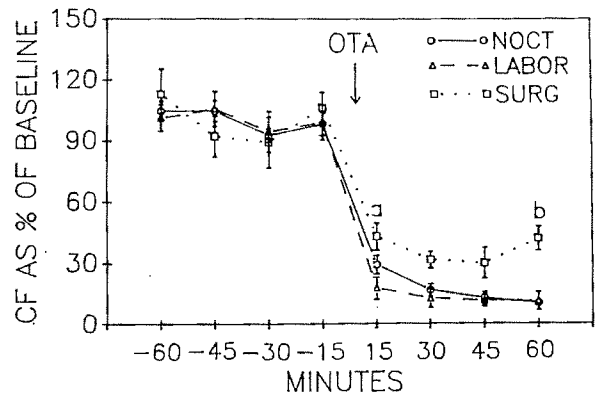


Fig. 6. This figure compares the contractile forces as a percent of baseline (see Fig. 5 for explanation) for nocturnal, labor, and surgery-related uterine activity. Uterine activity was diminished significantly (*asterisk*, $p < 0.05$) within 15 minutes for all study groups when compared with baseline. However, percent contractile force in the surgery group was decreased only by 60% 60 minutes after the intravenous bolus injection of the oxytocin antagonist, and this was statistically different (*b*, $p < 0.05$) from the nocturnal and labor activity, which was diminished by 90%. *OTA*, Oxytocin antagonist. *NOCT*, Nocturnal. *SURG*, Surgery. $N = 47$, $N = 10$, and $N = 7$ for nocturnal, labor, and surgery, respectively. Values are mean ± SEM. *a*, Different from the labor group ($p < 0.05$) but not the nocturnal group ($p > 0.05$).

prostaglandins) are not important for creating the appropriate environment or mediating oxytocin action but that oxytocin is directly responsible for controlling this uterine activity. The uterine activity may be a consequence of the change in uterine sensitivity to oxytocin, an increased release of oxytocin, or both. Such changes have been shown to occur with 24-hour rhythms in the rhesus monkey[12, 13] and baboon.[14] Also, the studies with the rhesus monkey[13] have shown that an oxytocin antagonist, different from the one in the present study, inhibits nocturnal uterine activity in this species. The fact that the oxytocin antagonist in the present study inhibits not only nocturnal uterine activity but also activity related to labor supports the concept that oxytocin is also an important regulator of this type of uterine activity. This is an important concept since the literature on humans has been ambiguous as to the stage of pregnancy or labor (first or second) in which oxytocin plays a role.[5-8] Assuming that the baboon is an appropriate model for human pregnancy, these data indicate that oxytocin is probably an important regulator of spontaneous nocturnal and labor uterine contractions in humans. These results support the studies showing that ethanol inhibits labor by suppressing oxytocin release.[15, 16]

Second, because of the physiologic similarity between baboons and humans, it seems likely that the oxytocin antagonist will inhibit spontaneous uterine actvity in humans. This assumption is supported by reports in the literature that a structurally different oxytocin antagonist can inhibit uterine activity of preterm labor in humans.[17] Thus our own data plus those in the literature imply that our oxytocin antagonist has the potential of inhibiting preterm labor in humans.

The fact that the oxytocin antagonist was not as effective in suppressing uterine contractile activity immediately after the operation when compared with spontaneous nocturnal and labor uterine contractions was not unexpected. It is likely that trauma to the uterus related to operation releases factors, such as prostaglandins, and these generate the uterine contractions. Under these conditions the generator for uterine contractions is local. However, since oxytocin release can

be induced reflexively (the Ferguson reflex[18]) by uterine contractions, one might expect oxytocin to be released and to contribute to this uterine activity. This is supported by the observation that the oxytocin antagonist appears to suppress uterine activity, but not totally eliminate it, when given immediately after operation. This is in contrast to the effect of the oxytocin antagonist on nocturnal uterine contractions and activity related to labor that is usually totally suppressed.

In our studies with the rat[9] the oxytocin antagonist disrupts labor, mainly by delaying its progress, but it does not appear to be as effective in inhibiting spontaneous uterine contractions as it is in the primate. Thus the oxytocin antagonist appears to be more efficacious in the primate than in the rat. In addition, the rat often showed incidences of diuresis (polydypsia and polyuria) while the baboons showed no apparent evidence of this potential side effect. Although this side effect is of concern, clinically it can be compensated by increasing water uptake. The opposite effect, antidiuresis, would be of greater concern.

The observation in Fig. 3 that oxytocin could reinitiate labor within 1 hour after oxytocin antagonist administration is important; if the oxytocin antagonist is used clinically, there would be occasions when labor needs to be reinitiated after the patient is evaluated. This observation suggests that within 1 hour, and perhaps less, the uterus will respond to exogenous oxytocin. Although not shown, the uterus will respond to exogenous prostaglandin $F_{2\alpha}$ and E_2 in the presence of oxytocin antagonist. Thus this is another potential means of reinitiating labor after oxytocin antagonist administration.

We have observed that the mother can express milk and the newborns can suckle within hours after delivery even though the oxytocin antagonist was given to the mother the day of or just before delivery. Therefore, even though the oxytocin antagonist can inhibit milk let-down,[9] this effect appears to be short-lived and should not interfere with nursing within several hours after delivery. However, this may not be the situation if the oxytocin antagonist is infused for long periods of time rather than given as intravenous bolus injections as in the present study.

Finally, it should be emphasized that the oxytocin antagonist has both antioxytocin and anti-antidiuretic hormone activity. Thus, although it seems likely that the oxytocin antagonist inhibits uterine activity by blocking endogenous oxytocin, it may be acting by blocking antidiuretic hormone as well. There are reports in the literature suggesting a role for antidiuretic hormone in labor.[19] Further studies are needed to assess this possibility.

In summary, we have shown that an oxytocin antagonist is a potent inhibitor of nocturnal uterine contraction and contraction related to labor in the baboon. These data suggest that oxytocin is the primary regulator of spontaneous nocturnal and labor uterine contraction during pregnancy in the baboon. Because of the physiologic similarity between humans and baboons the data imply that oxytocin is probably a primary regulator of uterine contractions in humans, and thus the oxytocin antagonist has the potential of inhibiting uterine contractions of preterm labor in humans.

We are indebted to Drs. Peter Nathanielsz, Department of Physiology, College of Veterinary Medicine, Cornell University, Ithaca, N.Y., and Raymond Stark, Department of Pediatrics, College of Physicians and Surgeons of Columbia University, New York, for allowing us to visit their laboratories and observe and discuss techniques required for establishing the tethered baboon model. In addition, we would like to thank Drs. Scott Walsh, Department of Obstetrics and Gynecology, Medical School of Virginia, Richmond, and George Haluska, Oregon Regional Primate Research Center, Portland, for additional advice in developing this model.

We thank Joanne McNeal and Denise Almahdi for helping with the baboon surgery and Irma Lopez for assistance in the manuscript preparation. Also we acknowledge the Scientific Computer Workstation and the Biostatistics Facility of the Research Resources Center, University of Illinois at Chicago, which provided the equipment and assistance necessary to conduct these computations.

REFERENCES

1. Fuchs A-R. The role of oxytocin in parturition. In: Huszar G, ed. The physiology and biochemistry of the uterus in pregnancy and labor. Boca Raton: CRC Press, 1986:163-83.
2. Mitchell MD. Prostaglandins during pregnancy and the perinatal period. J Reprod Fertil 1981;62:305-15.
3. Walsh SW. 5-Hydroxyeicosatetraenoic acid, leukotriene C_4 and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor. Am J Obstet Gynecol 1989; 161:1352-60.
4. Turnbull AC, Patten PT, Flint APF, Keirse MJNC, Jeremy JY, Anderson ABM. Significant fall in progesterone and rise in oestradiol levels in human peripheral plasma before onset of labor. Lancet 1974;1:101-3.
5. Dawood MY, Ylikorkala O, Trivedi D, Fuchs F. Oxytocin in maternal circulation and amniotic fluid during pregnancy. J Clin Endocrinol Metab 1979;49:429-34.
6. Leake RD, Weitzman RE, Glatz TH, Fisher DA. Plasma oxytocin concentrations in men, nonpregnant women, and pregnant women before and during spontaneous labor. J Clin Endocrinol Metab 1981;53:730-3.
7. Dawood MY, Raghavan KS, Pociask C. Radioimmunoassay of oxytocin. J Endocrinol 1978;76:261-70.
8. Padayachi T, Norman RJ, Dhavaraj K, Kemp M, Joubert SM. Serial oxytocin levels in amniotic fluid and maternal plasma during normal and induced labour. Br J Obstet Gynaecol 1988;95:888-93.
9. Wilson L, Parsons MT, Ouano L, Flouret G. A new tocolytic agent: development of an oxytocin antagonist for inhibiting uterine contractions. Am J Obstet Gynecol 1990;163:195-202.
10. Flouret G, Brieher W, Mahan K, Wilson L. Oxytocin an-

tagonist featuring D-tryptophan at position 2. In: Bayer E, Jung G, eds. Peptides 1988. New York: Walter DeGruyter 1989:549-51.
11. BMDP statistical software manual. Dixon WJ, ed. 1988 vol 2.
12. Honnebier MBO, Myers T, Figueroa JP, Nathanielsz PW. Variation in myometrial response to intravenous oxytocin administration at different times of the day in the pregnant rhesus monkey. Endocrinology 1989;125:1498-503.
13. Honnebier MBO, Figueroa JP, Rivier J, Vale W, Nathanielsz P. Studies in the role of oxytocin in late pregnancy in the rhesus monkey. J Dev Physiol 1989;12:225-32.
14. Morgan M, Silavin S, Myers T, Fishburne J, Nathanielsz P. Profiles of maternal plasma oxytocin (OT) concentration in baboons in the last trimester of pregnancy [Abstract 25]. In: Uterine contractility: mechanisms of control. St. Louis: Serono Symposium, March 17-20, 1990.
15. Fuchs F, Fuchs AR, Poblete VF, Risk A. Effect of alcohol on threatened premature labor. AM J OBSTET GYNECOL 1967;99:627-37.
16. Lauersen NH, Wilson KH, Fuchs F. The inhibitory effect of ethanol on oxytocin-induced labor. J Reprod Med 1981;26:547-50.
17. Akerlund M, Stromberg P, Hauksson A, et al. Inhibition of uterine contractions of premature labour with an oxytocin analogue. Br J Obstet Gynaecol 1987;94:1040-4.
18. Ferguson JKW. A study of the motility of the intact uterus at term. Surg Gynecol Obstet 1946;73:356-66.
19. Ivanisevic M, Behrens O, Helmer H, Demarest K, Fuchs A-R. Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists. AM J OBSTET GYNECOL 1989;161:1637-43.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells

Douglas A. Kniss, PhD,[a] John Mershon, MD,[a] Hsing-Chih Su, BS,[b] Jiri Sonek, MD,[a] Richard H. Fertel, PhD,[b] Michael Waxman, BS,[a] Jay D. Iams, MD,[a] and Steven G. Gabbe, MD[a]

Columbus, Ohio

Human amnion cells synthesize and release prostaglandin E_2 in response to epidermal growth factor. The protein kinase C activator, phorbol 12-myristate, 13-acetate also stimulates amnion cell prostaglandin E_2 synthesis. We used a human amnion cell line (WISH) to conduct in vitro experiments to investigate a potential role of protein kinase C in the signal transduction pathway leading to epidermal growth factor–induced prostaglandin E_2 production. Pretreatment of cultured amnion cells with a low, nonstimulating dose of phorbol 12-myristate, 13-acetate potentiated the action of epidermal growth factor in causing prostaglandin E_2 production as measured by radioimmunoassay. The protein kinase C–selective inhibitor staurosporine inhibited epidermal growth factor–induced prostaglandin E_2 production, further suggesting a role for protein kinase C in epidermal growth factor action. Experiments were conducted in which amnion cells were rendered protein kinase C–deficient by chronic exposure to phorbol ester, which has been shown to down-regulate the enzyme. In these cells, epidermal growth factor caused prostaglandin E_2 synthesis at levels comparable to native (non-protein kinase C–deficient) cells. We conclude that protein kinase C plays a more modulatory than direct role in the epidermal growth factor signal transduction cascade that leads to prostaglandin E_2 production by amnion cells. We propose a bifurcating transduction scheme in which, under conditions of protein kinase C inactivation, epidermal growth factor alone causes prostaglandin E_2 synthesis. When protein kinase C is activated by as yet unknown endogenous substances, the epidermal growth factor responsiveness of the amnion cells is greatly enhanced. This pathway could have important implications in a feed-forward mechanism regulating the level of prostaglandin E_2 production during the onset of labor. (Am J Obstet Gynecol 1990;163: 1883-90.)

Key words: Amnion cells, cell culture, prostaglandin E_2, EGF, protein kinase C

It is now well established that prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) are the active mediators of two key parturitional events, cervical ripening and myometrial contractions. Arachidonic acid and prostaglandins accumulate in amniotic fluid in association with the onset of labor at term.[1] In addition, Romero et al.[2] have reported the presence of high levels of PGE_2 and $PGF_{2\alpha}$ in amniotic fluid samples obtained from women in preterm labor complicated by intraamniotic infection. The human amnion membrane is thought to be a major contributor to the amniotic fluid pool of PGE_2, and as such, the fetal membranes may play an important role in the onset of labor.[3, 4] However, it is not yet clear which signal molecules govern amnion prostaglandin production. One candidate molecule is epidermal growth factor. Because epidermal growth factor levels in human amniotic fluid increase steadily from 30 to 40 weeks' gestation and epidermal growth factor stimulates the production of PGE_2 in primary cultures of human amnion cells, investigators proposed that this molecule plays an important role in perinatal development and parturition.[5-7] Moreover, epidermal growth factor has been suggested to be derived, at least in part, from the fetal kidney, thus supporting the hypothesis that the signal triggering labor in women emanates from the fetus.[5, 8, 9] Epidermal growth factor is thus an attractive molecule for further studies of the regulation of amnion cell prostaglandin production.

There is evidence that activation of protein kinase C by tumor-promoting phorbol esters causes PGE_2 synthesis in amnion cells, and calcium-sensitive/phospholipid-dependent protein kinase activity has been characterized in amnion tissue.[10-12] Because epidermal growth factor in other cell systems acts at least partially via a protein kinase C–mediated mechanism, we sought to determine whether protein kinase

From the Departments of Obstetrics and Gynecology (Division of Maternal-Fetal Medicine)[a] and Pharmacology,[b] The Ohio State University College of Medicine.
Supported in part by a grant from the Bremer Foundation at The Ohio State University School of Medicine.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: D. A. Kniss, PhD, Department of Obstetrics and Gynecology, The Ohio State University, College of Medicine, 1654 Upham Dr., Columbus, OH 43210.
6/6/24768

C activation was involved in epidermal growth factor–induced PGE_2 synthesis in human amnion cells.[13-16] For our studies, we used the established human amnion cell line (WISH) as a model system.[17] Harris et al.[18] have reported that WISH cells respond to phorbol esters and epidermal growth factor by producing PGE_2, as do primary cultures of human amnion cells.

WISH cells have the added advantages of being more stable and providing a more homogeneous population of cells than primary amnion cell cultures. Our data suggest that epidermal growth factor may exert part of its PGE_2 synthesizing action via a protein kinase C–associated pathway.

Material and methods

Cell cultures. The established human amnion cell line (WISH) obtained from the American Type Culture Collection (ATCC, CCL25) was maintained in Ham's F12/Dulbecco's modified Eagle's medium (1:1) supplemented with 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 50 μg/ml gentamicin sulfate, and 10% newborn calf serum. The cells were subcultured once weekly, and, when used for experiments, were seeded into NUNC 24-well plates (2×10^5 cells per well) or Corning 12-well plates (5×10^5 cells per well). After 2 days' growth, the cells were 80% to 90% confluent; they were then rinsed with serum-free F12/Dulbecco's modified Eagle's medium and treated with test substances. The cells were stimulated for 4 to 12 hours. The incubation media were collected and analyzed for the presence of PGE_2 by specific radioimmunoassay (RIA).

PGE_2-specific radioimmunoassay. Media collected from stimulated WISH cell cultures were analyzed for the presence of PGE_2 by specific RIA with anti-PGE_2 prepared by inoculating domestic chickens with PGE_2-keyhole limpet hemocyanin conjugate. The full protocol for the preparation of this antibody has been published elsewhere.[19] The percent cross-reactivity for this antibody is as follows: arachidonic acid, 0.01%; PGA, 0.16%; PGD_2, 1.6%; PGE_2, 100%; $PGF_{1\alpha}$, 3.2%; $PGF_{2\alpha}$, 0.75%; 6-keto-$PGF_{1\alpha}$, 0.05%; thromboxane B_2, 0.41%.

Tritiated phorbol 12,13-dibutyrate binding studies. WISH cells were seeded into Costar 12-well plates at 2.5×10^5 cells per well in F12/Dulbecco's modified Eagle's medium + 10% newborn calf serum. One day after plating the cells were exposed to 1 μmol/L phorbol 12,13-dibutyrate or 0.001% dimethyl sulfoxide in the same media for 48 hours. After exposure to phorbol 12,13-dibutyrate, the cells were rinsed five times with warm Ham's F10 supplemented with 15 mmol/L Hepes (pH 7.2) and 1 mg/ml bovine serum albumin. The cells were then exposed to tritiated phorbol 12,13-dibutyrate (1 to 50 nmol/L) for 60 min at 37° C. Nonspecific binding was assessed by treating companion wells with 1 μmol/L phorbol 12-myristate, 13-acetate during the binding interval and subtracting the amount of ligand bound in the presence of phorbol 12-myristate, 13-acetate from total binding. After the binding period the cells were rinsed extensively with F10 medium and then solubilized with 1% Triton X-100/1% sodium dodecyl sulfate. Radioactivity was counted by liquid scintillation spectrometry.

Protein kinase C down-regulation studies. To down-regulate protein kinase C in the WISH cells, they were treated 1 day after seeding with 1 μmol/L phorbol 12,13-dibutyrate or 0.001% dimethyl sulfoxide as described. Two days after phorbol treatment the cells were rinsed extensively to remove the phorbol 12,13-dibutyrate and were then treated with epidermal growth factor, phorbol 12-myristate, 13-acetate, or vehicle. Preliminary experiments with tritiated phorbol 12,13-dibutyrate as a tracer demonstrated that with extensive rinsing 95% of the phorbol 12,13-dibutyrate could be removed from the cells.

Statistical analysis. Each experiment was performed with six replicates per condition. In all cases the experiments were repeated a minimum of two times. The data were analyzed with Student's t test (two-tailed) to determine statistically significant differences between control and treatment groups.

Material. All tissue culture reagents were obtained from Gibco/BRL (Grand Island, N.Y.). Phorbol esters and dimethyl sulfoxide were purchased from Sigma Chemical Co. (St. Louis). Murine epidermal growth factor was from Collaborative Research (Bedford, Mass.). Staurosporine was obtained from Boehringer Mannheim (Indianapolis). Tritiated PGE_2 was purchased from Amersham (Arlington Heights, Ill.).

Results

Effect of phorbol esters on PGE_2 synthesis in amnion cells. To determine whether protein kinase C is associated with PGE_2 production in WISH cells, we incubated cells with phorbol 12-myristate 13-acetate, phorbol 12,13-dibutyrate, or 4α-phorbol at concentrations ranging from 10^{-7} to 10^{-10} mol/L. Our results (Fig. 1) indicate that, at a concentration of 10^{-7} mol/L, phorbol 12-myristate, 13-acetate caused an eighteenfold increase in PGE_2 production versus control ($p < 0.001$). Phorbol 12,13-dibutyrate, a less potent analog of phorbol 12-myristate, 13-acetate, caused a tenfold increase in PGE_2 production ($p < 0.01$). In contrast, the inactive phorbol, 4α-phorbol, had no effect on PGE_2 production. On the basis of this experiment, we concluded that active phorbol esters stimulate PGE_2 production in WISH cells.

Synergistic effect of epidermal growth factor and phorbol 12-myristate, 13-acetate on PGE_2 synthesis. To determine whether epidermal growth factor and phorbol 12-myristate, 13-acetate act via a similar mech-

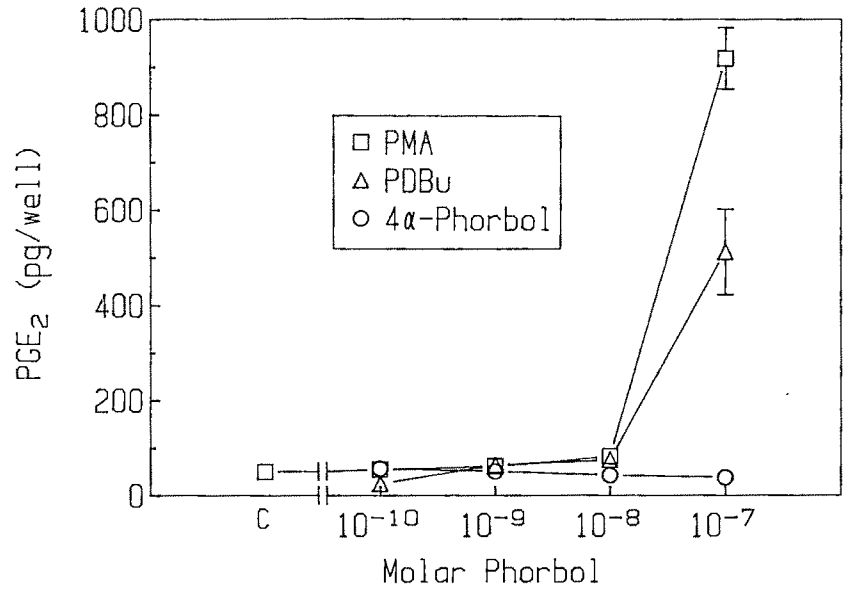


Fig. 1. Phorbol dose-response. WISH cells were seeded into 24-well plates at 2×10^5 cells/well and grown for 2 days. The cells were then treated with either phorbol 12-myristate, 13-acetate, phorbol 12,13-dibutyrate, or 4α-phorbol at concentrations ranging from 10^{-7} to 10^{-10} mol/L in F12/Dulbecco's modified Eagle's medium plus 10% newborn calf serum. Control cultures were incubated with 0.01% dimethyl sulfoxide. After 12 hours of stimulation, the media were collected and analyzed for the presence of PGE_2 by radioimmunoassay. The data, expressed as picograms of PGE_2 produced per well per 12 hours, represent the mean ± SEM of six replicates per condition and are representative of two individual experiments. Student's *t* test revealed significant differences between 10^{-7} mol/L phorbol 12-myristate, 13-acetate and control and 10^{-7} mol/L phorbol 12,13-dibutyrate and control, $p < 0.001$. All other phorbol concentrations were not significantly different from controls ($p > 0.05$). (*C*, Control; *squares*, phorbol 12-myristate, 13-acetate; *triangles*, phorbol 12,13-dibutyrate; *circles*, 4α-phorbol.)

anism, we studied the effect of a stimulatory concentration of epidermal growth factor (10 ng/ml) on amnion cell PGE_2 synthesis in the presence or absence of a substimulatory dose of phorbol 12-myristate, 13-acetate (1 nmol/L). Our results (Fig. 2) indicate that 10 ng/ml of epidermal growth factor caused a tenfold increase in PGE_2 production by the WISH cells after a 4-hour incubation period ($p < 0.001$). At a concentration of 1 nmol/L, phorbol 12-myristate, 13-acetate had a minimal effect on PGE_2 production. This is in accord with the previous dose-response experiments with phorbol 12-myristate, 13-acetate (Fig. 1). However, when epidermal growth factor was coincubated with 1 nmol/L phorbol 12-myristate, 13-acetate, there was a fifteenfold enhancement of PGE_2 synthesis over control, and a 66% enhancement over epidermal growth factor alone ($p < 0.001$). These results suggest that a concentration of phorbol 12-myristate, 13-acetate that itself is not stimulatory is capable of enhancing the effect of epidermal growth factor. To further test this idea, we preincubated the cells with phorbol 12-myristate, 13-acetate, removed the phorbol by thorough washing of the cells, and then treated the cells with a stimulatory dose of epidermal growth factor. Our results (Fig. 3) indicate that preincubation with phorbol 12-myristate, 13-acetate alone had little effect on PGE_2 production. In contrast, the epidermal growth factor response was stimulated nearly threefold by prior exposure of the cells to phorbol 12-myristate, 13-acetate ($p < 0.001$). The inactive phorbol, 4α-phorbol, which does not activate protein kinase C, had no effect on the epidermal growth factor response (data not shown). On the basis of these studies, we conclude that pretreatment or coincubation with a low dose of phorbol 12-myristate, 13-acetate (1 nmol/L) significantly enhances the responsiveness of the amnion cells to epidermal growth factor.

Effect of protein kinase C inhibitors on epidermal growth factor–induced PGE_2 synthesis. To test further the relationship between epidermal growth factor and protein kinase C activation, WISH cell cultures were pretreated with the protein kinase C inhibitor staurosporine for 30 minutes before epidermal growth factor exposure. Preliminary experiments demonstrated that staurosporine was able to inhibit phorbol 12-myristate, 13-acetate–induced PGE_2 synthesis in a dose-dependent manner (data not shown). Our results (Fig. 4) indicate that staurosporine inhibited epidermal growth factor–induced PGE_2 production in a dose-dependent manner. A concentration of 5 nmol/L stau-

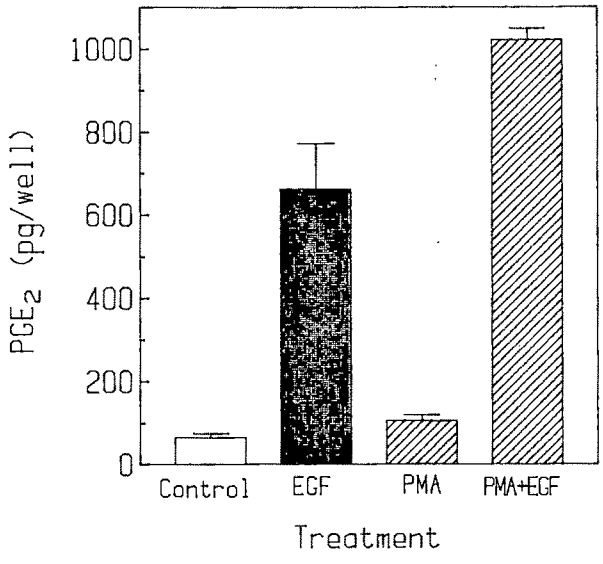


Fig. 2. Phorbol 12-myristate, 13-acetate enhancement of epidermal growth factor–induced PGE_2 synthesis. WISH cell cultures were seeded into 24-well plates at 2×10^5 cells/well and grown for 2 days. The cells were then treated with epidermal growth factor (10 ng/ml) in the presence of 1 nmol/L phorbol 12-myristate, 13-acetate. Control cultures received 0.0001% dimethyl sulfoxide. After a 4-hour incubation period, PGE_2 in the culture media was measured by radioimmunoassay. The data represent mean ± SEM of six replicates per condition and are representative of two separate experiments. ($p < 0.001$, epidermal growth factor vs phorbol 12-myristate, 13-acetate + epidermal growth factor.)

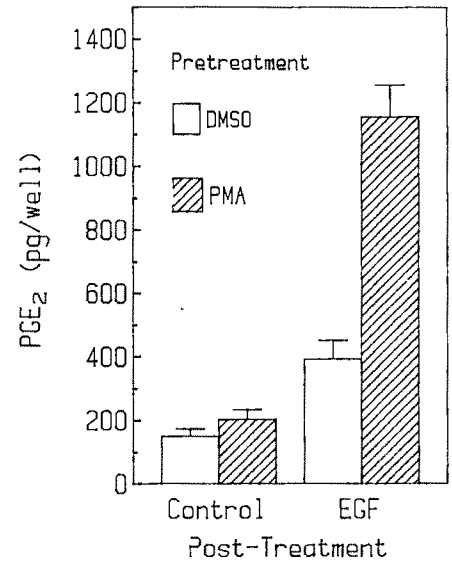


Fig. 3. Phorbol 12-myristate, 13-acetate preincubation augments the epidermal growth factor response. WISH cells were seeded into 24-well plates at 2×10^5 cells/well in F12/Dulbecco's modified Eagle's medium + 10% newborn calf serum and grown for 2 days. The cells were then fed fresh medium (serumfree F12/Dulbecco's modified Eagle's medium) containing phorbol 12-myristate, 13-acetate (0.1 μmol/L) or dimethyl sulfoxide (0.01%) for 4 hours. After the preincubation period the cells were rinsed thoroughly with serumfree F12/Dulbecco's modified Eagle's medium and then exposed to epidermal growth factor (10 ng/ml) in F12/Dulbecco's modified Eagle's medium + 10% newborn calf serum. Control cultures received medium alone. After an additional 4-hour incubation period, PGE_2 in the media was measured by radioimmunoassay. The data represent mean ± SEM of six replicates per condition and are representative of four similar experiments.

rosporine caused a 43% reduction in PGE_2 synthesis compared with epidermal growth factor alone. At 100 nmol/L staurosporine, PGE_2 synthesis in response to epidermal growth factor was reduced by 65%. Staurosporine alone had no effect on PGE_2 production (data not shown). These results provide further evidence for the role of protein kinase C in the prostaglandin synthetic pathway.

Effect of protein kinase C down-regulation on epidermal growth factor–induced PGE_2 synthesis. If protein kinase C is the only pathway by which epidermal growth factor can stimulate the production of PGE_2 in amnion cells, then down-regulation of the enzyme should diminish or abolish the epidermal growth factor response. To test this possibility, we preincubated the cells with phorbol 12,13-dibutyrate (1 μmol/L) for 48 hours to down-regulate protein kinase C. Previous studies have shown that long-term exposure to a high dose of phorbol ester is sufficient to eliminate nearly all the protein kinase C.[19, 20] To verify the down-regulation phenomenon, we used tritiated phorbol 12,13-dibutyrate to evaluate the presence of phorbol binding sites (i.e., protein kinase C) after chronic phorbol exposure. As predicted, there was an 85% reduction in phorbol binding sites in chronically phorbol-treated cells compared with vehicle-treated (dimethyl sulfoxide) controls (Fig. 5, inset). On the basis of this study, we conducted experiments to examine the effect of protein kinase C down-regulation on epidermal growth factor–induced PGE_2 synthesis. When the cells were chronically exposed to 1 μmol/L phorbol 12,13-dibutyrate and then challenged with phorbol 12-myristate, 13-acetate 48 hours later, there was a complete diminution of phorbol 12-myristate, 13-acetate–induced PGE_2 production (Fig. 5, $p > 0.01$ for dimethyl sulfoxide vs phorbol 12,13-dibutyrate pretreatment in the phorbol 12-myristate, 13-acetate–treated group). In contrast, when protein kinase C "deficient" cells were challenged with epidermal growth factor, there was no apparent reduction in PGE_2 synthesis. In fact, there was a modest, albeit statistically significant, enhancement of epidermal growth factor–induced PGE_2 production (Fig. 5, $p < 0.05$, for dimethyl sulfoxide vs phorbol 12,13-dibutyrate pretreatment in epidermal growth factor–treated group). These data suggest that, although protein kinase C is likely involved in epidermal growth factor–induced PGE_2 production by am-

nion cells, a protein kinase C–*independent* pathway may also exist.

Comment

In this study we sought to determine whether epidermal growth factor–induced PGE_2 synthesis in cultured human amnion cells was mediated via a protein kinase C–dependent pathway. For our studies we used the immortal human amnion cell line (WISH) as a model system. A previous report by Harris et al.[18] provided evidence that WISH cells respond to phorbol esters and epidermal growth factor by producing eicosanoids (primarily PGE_2). Similar results have been reported by several laboratories using primary amnion cell cultures.[10, 12, 21, 22] In our hands, the WISH cell line has been much more stable than primary amnion cell cultures prepared from fetal membranes obtained at cesarean section. Thus we believe that the WISH cells offer another model system for studies of the mechanisms governing PGE_2 production in amnion cells.

Our studies, in which a low, nonstimulating concentration of phorbol 12-myristate, 13-acetate augmented the epidermal growth factor response, suggest that protein kinase C activation may be involved in the transduction pathway leading to epidermal growth factor–induced prostaglandin synthesis in amnion cells. A recent report by Sanders and Myatt[23] has clearly established a role for protein kinase C in the amnion cell prostaglandin cascade by showing that several putative inhibitors of protein kinase C abolished phorbol 12-myristate, 13-acetate–induced PGE_2 production. Our results are in accordance with the findings of Margolis et al.,[24] who reported that, in rat kidney glomerular mesangial cells, epidermal growth factor and phorbol esters interacted synergistically to stimulate arachidonic acid liberation and PGE_2 production. In addition, our studies showing that the inactive phorbol ester 4α-phorbol failed to synergize with epidermal growth factor provide further evidence that activation of protein kinase C by active phorbols (i.e., phorbol 12-myristate, 13-acetate) is required for potentiation of the epidermal growth factor signal. To test this concept further, we used the selective protein kinase C inhibitor staurosporine. Although staurosporine shows some inhibitory activity for cyclic adenosine monophosphate–dependent and calcium/calmodulin-dependent protein kinases, it appears to be fairly selective for protein kinase C, especially at lower concentrations (e.g., 5 to 50 nmol/L).

Staurosporine reduced epidermal growth factor–induced PGE_2 synthesis at concentrations as low as 5 nmol/L. However, complete inhibition of the epidermal growth factor response was not achieved even at 100 nmol/L staurosporine. This result suggests the possibility that protein kinase C activation is not an absolute requirement for epidermal growth factor–induced PGE_2 production in amnion cells, but plays a more modulatory role. In an attempt to confirm the role of protein kinase C in epidermal growth factor–induced PGE_2 synthesis, we performed protein kinase C down-regulation experiments. WISH cells were exposed chronically to a high concentration of phorbol 12,13-dibutyrate. The more hydrophilic phorbol 12,13-dibutyrate was chosen for preexposure because preliminary studies had established that it was more easily washed from the cells than was phorbol 12-myristate, 13-acetate (data not shown). Phorbol 12-myristate, 13-acetate, which is more lipophilic, appears to be more tightly sequestered within the cell membrane, thus making washout more difficult. After 48 hours, by use of radioligand-binding techniques, we demonstrated an 85% reduction in tritiated phorbol 12,13-dibutyrate binding sites, suggesting a net loss of protein kinase C molecules (i.e., protein kinase C–deficient cells).[20, 25] As expected, when the cells were subsequently challenged with phorbol 12-myristate, 13-acetate, they were unable to produce PGE_2. In contrast, epidermal growth factor stimulated PGE_2 production in downregulated cells at levels comparable with cells treated with vehicle alone. In fact, there was actually a slight augmentation of the

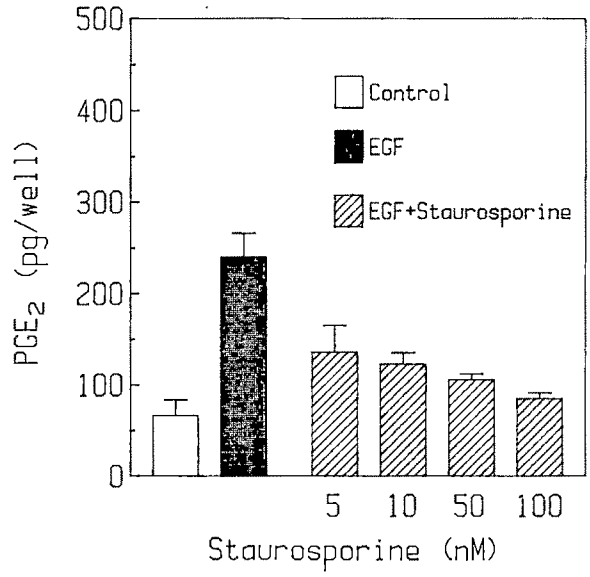


Fig. 4. Staurosporine inhibits EGF–induced PGE_2 synthesis. WISH cells were seeded into 24-well plates at 2×10^5 cells/well in F12/Dulbecco's modified Eagle's medium + 10% newborn calf serum and grown for 2 days. The cells were then fed with fresh medium containing 5 to 100 nmol/L staurosporine. Control cultures received 0.01% dimethyl sulfoxide. After a 30-minute preincubation with staurosporine the cells were treated with epidermal growth factor (10 ng/ml) for 4 hours. Media were collected and analyzed for the presence of PGE_2 by radioimmunoassay. The data represent mean ± SEM of six replicates per condition and are representative of three similar experiments.

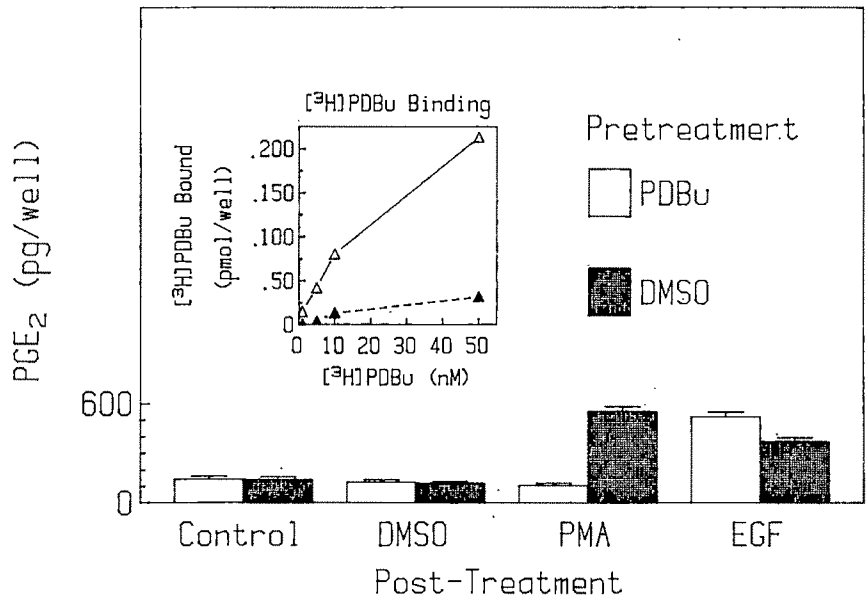


Fig. 5. Phorbol ester–induced protein kinase C down-regulation. WISH cells were seeded into 24-well plates at 2×10^5 cells/well in F12/Dulbecco's modified Eagle's medium + 10% newborn calf serum and grown for 1 day. The cells were then exposed to phorbol 12,13-dibutyrate (1 μmol/L) or dimethyl sulfoxide (0.01%). After 48 hours the cells were rinsed thoroughly with serum-free F12/Dulbecco's modified Eagle's medium and then fed fresh F12/Dulbecco's modified Eagle's medium + 10% newborn calf serum. The cells were exposed to epidermal growth factor (10 ng/ml), phorbol 12-myristate, 13-acetate (0.1 μmol/L), or dimethyl sulfoxide (0.01%). After a 4-hour stimulation period the media were collected and analyzed for the presence of PGE_2 by radioimmunoassay. The data represent mean ± SEM of six replicates per condition and are representative of two similar experiments. *Inset,* Tritiated phorbol 12,13-dibutyrate binding in protein kinase C downregulated cells. WISH cells were seeded into 12-well plates at 2.5×10^5 per well in F12/Dulbecco's modified Eagle's medium + 10% newborn calf serum and grown for 1 day. The cells were then exposed to phorbol 12,13-dibutyrate (1 μmol/L) or dimethyl sulfoxide (0.01%). After 48 hours of phorbol exposure, the cells were then rinsed with F10 + 15 mmol/L HEPES (pH 7.2) and 1 mg/ml bovine serum albumin. Tritiated phorbol 12,13-dibutyrate binding was conducted with 1 to 50 nmol/L radioligand for 1 hour at 37° C as described in Material and methods. The data represent picomoles per well of specifically bound tritiated phorbol 12,13-dibutyrate (triplicates determinations per condition) and are representative of two similar experiments. (*Solid symbols,* phorbol 12,13-dibutyrate-treated; *open symbols,* dimethyl sulfoxide-treated).

epidermal growth factor response. These results argue strongly that protein kinase C activation is not an absolute requisite for the epidermal growth factor response in amnion cells.

Margolis et al.[24] concluded, on the basis of protein kinase C down-regulation experiments, that protein kinase C did not mediate the effects of epidermal growth factor in rat kidney cells. They reported that long-term phorbol treatment failed to abolish epidermal growth factor–induced PGE_2 synthesis in the cells. However, they did find a decrease, albeit incomplete, in the epidermal growth factor/phorbol 12-myristate, 13-acetate synergism in the absence of phorbol 12-myristate, 13-acetate pretreatment (see Fig. 5 and reference 24). Although the down-regulation experiments are instructive, it must be stressed that the failure to abolish the epidermal growth factor response in protein kinase C–deficient cells is not de facto evidence for the lack of a role for protein kinase C in the epidermal growth factor transduction pathway.

Our studies showing that phorbol 12-myristate, 13-acetate markedly potentiated the epidermal growth factor response and staurosporine inhibited it provide compelling evidence that protein kinase C plays a significant modulatory role in epidermal growth factor–induced PGE_2 synthesis. Modulation of the epidermal growth factor transduction pathway by protein kinase C could occur at one of several sites. First, it is possible that phorbol-induced modulation of the epidermal growth factor receptor supersensitizes the amnion cells to epidermal growth factor. Such modulation of the epidermal growth factor receptor in WISH cells has been reported by Karasaki et al.[26] Another putative target for protein kinase C modulation of the epidermal growth factor transduction mechanism is at the level of a guanine nucleotide–binding regulatory protein (G

protein), which is thought to be interposed between the epidermal growth factor receptor and the effector system(s) with which it interacts.[27, 28] Protein kinase C–mediated G protein phosphorylation has indeed been demonstrated in other systems.[29, 30] A third possible protein kinase C target is phospholipase A_2, cyclooxygenase, or both. Experiments are currently in progress in our laboratory to define the role of protein kinase C in the liberation of arachidonic acid by phospholipase A_2 in amnion cells. Furthermore, it is possible that protein kinase C augments the induction of prostaglandin H_2 synthase by epidermal growth factor.[22]

In light of our potentiation and inhibitor studies, it is premature to discount a modulatory role for protein kinase C in epidermal growth factor–induced PGE_2 production solely on the basis of the failure of protein kinase C down-regulation to abrogate the epidermal growth factor response. It is more likely that a complex modulatory event(s) exists at one or more of the sites described to augment the epidermal growth factor response. We propose a bifurcating mechanism in which epidermal growth factor, under conditions of protein kinase C inactivation, is capable of inducing PGE_2 production alone. Under the influence of an as yet unknown endogenous activator of protein kinase C, however, the sensitivity of the amnion cells to epidermal growth factor is greatly augmented, thus leading to a markedly enhanced response.

We thank Dr. Chad Friedman for his constructive comments regarding the manuscript.

REFERENCES

1. Keirse MJNC, Hicks BR, Mitchell MD, Turnbull AC. Increase of the prostaglandin precursor, arachidonic acid, in amniotic fluid during spontaneous labor. Br J Obstet Gynaecol 1977;84:937-40.
2. Romero R, Emanian M, Wan M, Quintero R, Hobbins JC, and Mitchell MD. Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor. Am J Obstet Gynecol 1987;157:1461-7.
3. Bleasdale JE, DiRenzo GC. Fetal influence on human parturition. In: Rodeck CH, ed. Fetal medicine. Oxford, England: Blackwell Scientific, 1989 vol 1:242.
4. Liggins GC. Initiation of labour. Biol Neonate 1989; 55:366-75.
5. Haigh R, D'Souza SW, Micklewright L, et al. Human amniotic urogastrone (epidermal growth factor) and fetal lung phospholipids. Br J Obstet Gynaecol 1989;96:1710-78.
6. Casey ML, Mitchell MD, MacDonald PC. Epidermal growth factor-stimulated prostaglandin E_2 production in human amnion cells: specificity and nonesterified arachidonic acid dependency. Mol Cell Endocrinol 1987;53:169-76.
7. Zakar T, Olson DM. The action of epidermal growth factor on human amnion prostaglandin E_2 output. Can J Physiol Pharmacol 1988;66:769-75.
8. Scott SM, Buenaflor FF, Orth DN. Immunoreactive human epidermal growth factor concentrations in amniotic fluid, umbilical artery and vein serum, and placenta in full-term and preterm infants. Biol Neonate 1989;56:246-51.
9. Casey ML, MacDonald PC. The role of a fetal-maternal paracrine system in the maintenance of pregnancy and the initiation of parturition. In: Jones CT, ed. Fetal and neonatal development. Research in perinatal medicine. IV. Ithaca, New York: Perinatology Press, 1988:521.
10. Lytton FD, Mitchell MD. Phorbol ester-induced stimulation of prostaglandin biosynthesis in human amnion cells. Biochim Biophys Acta 1988;959:399-401.
11. Okazaki T, Ban C, Johnston JM. The identification and characterization of protein kinase C activity in fetal membranes. Arch Biochem Biophys 1984;229:27-32.
12. Zakar T, Olson DM. Stimulation of human amnion prostaglandin E_2 production by activators of protein kinase-C. J Clin Endocrinol Metab 1988;67:915-23.
13. Decker SJ. Effects of epidermal growth factor and 12-O-tetradecanoylphorbol-13-acetate on metabolism of the epidermal growth factor receptor in normal human fibroblasts. Mol Cell Biol 1984;4:1718-24.
14. Whiteley B, Glaser L. Epidermal growth factor (EGF) promotes phosphorylation at threonine-654 of the EGF receptor: possible role of protein kinase C in homologous regulation of the EGF receptor. J Cell Biol 1986;103: 1355-62.
15. Earp HS, Hepler JR, Petch LA, et al. Epidermal growth factor (EGF) and hormones stimulate phosphoinositide hydrolysis and increase EGF receptor protein synthesis and mRNA levels in rat liver epithelial cells. Evidence for protein kinase C-dependent and -independent pathways. J Biol Chem 1988;263:13868-74.
16. Kaji H, Casnellie JE, Hinkle PM. Thyrotropin releasing hormone action in pituitary cells. Protein kinase C-mediated effects on the epidermal growth factor receptor. J Biol Chem 1988;263:13588-93.
17. Hayflick L. The establishment of a line (WISH) of human amnion cells in continuous cultivation. Exp Cell Res 1961;23:14-20.
18. Harris AN, Perlman M, Schiller SL, Romero R, Mitchell MD. Characterization of prostaglandin production in amnion-derived WISH cells. Am J Obstet Gynecol 1988;159:1385-9.
19. Fertel R, Yetiv JZ, Coleman MA, Schwarz RD, Greenwald JE, Bianchine JR. Formation of antibodies to prostaglandins in the yolk of chicken eggs. Biochem Biophys Res Commun 1981;102:1028-33.
20. Rodriguez-Pena A, Rozengurt E. Disappearance of Ca^{2+}-sensitive, phospholipid-dependent protein kinase activity in phorbol ester-treated 3T3 cells. Biochem Biophys Res Commun 1984;120;1053-9.
21. Mitchell MD. Epidermal growth factor actions on arachidonic acid metabolism in human amnion cells. Biochim Biophys Acta 1987;928:240-2.
22. Casey ML, Korte K, MacDonald PC. Epidermal growth factor stimulation of prostaglandin E_2 biosynthesis in amnion cells. Induction of prostaglandin H_2 synthase. J Biol Chem 1988;263:7846-54.
23. Sanders J, Myatt L. Regulation of prostaglandin E_2 synthesis in human amnion by protein kinase C. Prostaglandins 1990;39:355-63.
24. Margolis BL, Bonventre JV, Kremer SG, Kudlow JE, Skorecki KL. Epidermal growth factor is synergistic with phorbol esters and vasopressin in stimulating arachidonate release and prostaglandin production in renal glomerular mesangial cells. Biochem J 1988;249:587-92.
25. Ashendel CL. The phorbol ester receptor: a phospholipid-regulated protein kinase. Biochim Biophys Acta 1985;822:219-42.
26. Karasaki Y, Jaken S, Komoriya A, Zoon KC. Phorbol ester and interferon-γ modulation of epidermal growth factor receptors in human amniotic (WISH) cells. J Biol Chem 1989;264:6158-63.
27. Nair BG, Rashed HM, Patel TB. Epidermal growth factor stimulates rat cardiac adenylate cyclase through a GTP-binding regulatory protein. Biochem J 1989;264:563-71.

28. Teitelbaum I. The epidermal growth factor receptor is coupled to a phospholipase A_2-specific pertussis toxin-inhibitable guanine nucleotide-binding regulatory protein in cultured rat inner medullary collecting tubule cells. J Biol Chem 1990;265:4218-22.
29. Zick Y, Sagi-Eisenberg R, Pines M, Gierschik P, and Spiegel AM. Multisite phosphorylation of the α subunit of transducin by the insulin receptor kinase and protein kinase C. Proc Natl Acad Sci USA 1986;83:9294-7.
30. Crouch M, Lapetina E. A role for G_i in control of thrombin receptor-phospholipase C coupling in human platelets. J Biol Chem 1988;263:3363-71.

Lymphokine production during term human pregnancy: Differences between peripheral leukocytes and decidual cells

Donald J. Dudley, MD, Murray D. Mitchell, DPhil, Kathryn Creighton, BS, and D. Ware Branch, MD

Salt Lake City, Utah

Although the fetus is considered to be an "allograft," there is little information concerning the role of lymphokines in human pregnancy. Lymphokines are polypeptides secreted by stimulated lymphocytes that direct the immune response by enabling immune effector cells to communicate with each other. To characterize lymphokine production during normal human pregnancy, we isolated peripheral leukocytes and decidual lymphocyte-like cells from women undergoing repeat cesarean section at term. After stimulation with mitogen and paternal antigen for 24 hours, culture supernatants were assayed for granulocyte-macrophage colony-stimulating factor and interleukin-2 by enzyme-linked immunosorbent assay. There was no difference in the amount of interleukin-2 produced by stimulated peripheral and decidual cells. However, granulocyte-macrophage colony-stimulating factor production by stimulated decidual lymphocyte-like cells was significantly greater than granulocyte-macrophage colony-stimulating factor produced by peripheral lymphocytes. Decidual lymphocyte-like cells produced granulocyte-macrophage colony-stimulating factor both spontaneously and after stimulation with mitogen or paternal antigen, whereas peripheral leukocytes did not. This suggests that the decidua constitutes a distinct immunologic microenvironment. (Am J Obstet Gynecol 1990;163:1890-3.)

Key words: Pregnancy, lymphokines, granulocyte-macrophage colony-stimulating factor, interleukin-2

Our knowledge of the immunologic relationship between the mother and conceptus is incomplete. The conceptus appears to elicit a specific maternal immune response consisting of antibody production[1] and changes in maternal cellular immunity. This is detected as diminished proliferation of maternal peripheral blood leukocytes to irradiated paternal peripheral blood leukocytes in a mixed leukocyte culture.[2] However, some investigators believe that the important cellular immune responses to fetopaternal antigens occur in the decidua at the maternal-fetal interface.[3] It is currently thought that these decidual immune responses are beneficial to the survival of the conceptus.[4]

Cellular immune responses are characterized by the production of a variety of polypeptides known as lymphokines. Little information concerning the role of lymphokines in protecting the fetal allograft is available. Moreover, few studies have addressed the function of lymphoid cells in the human decidua. The purpose of our study was to characterize lymphokine production during normal human pregnancy, comparing the production of granulocyte-macrophage colony-stimulating factor and interleukine-2 by peripheral leukocytes with that of decidual lymphocyte-like cells. We hypothesized that the decidua constitutes a distinct immunologic environment that supports immune effector cells that produce lymphokines different from those produced by peripheral leukocytes.

Material and methods

Placentas were obtained aseptically from normal human pregnancies at term repeat cesarean section. The

From the Department of Obstetrics and Gynecology, University of Utah School of Medicine.
Supported by a grant from the Willard L. Eccles Charitable Foundation.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Donald J. Dudley, MD, Department of Obstetrics and Gynecology, University of Utah School of Medicine, 50 N. Medical Dr., Salt Lake City, UT 84132.
6/6/24761

membranes were isolated and rinsed in Dulbecco's modified Eagle's medium. A modification of the method described by Delvin et al.[5] was used to isolate decidual lymphocyte-like cells. The decidua adherent to the maternal side of the chorion laevae was removed by sharp dissection to yield a cell homogenate. To disperse the cells, the homogenate was mixed with 63 units of collagenase per milliliter of Dulbecco's modified Eagle's medium (100 ml total volume) at 37° C for 60 to 120 minutes. The cell homogenate was filtered through a gauze mesh to remove large debris. The cells then were centrifuged over Histopaque 1077 (Sigma, St. Louis) at 1000 *g* for 30 minutes to isolate the lymphocyte-like cell fraction. This fraction was aspirated and rinsed. The culture medium used throughout these experiments was RPMI with 1% Nutridoma-HU (Boehringer-Mannheim, Indianapolis) and antibiotics (100 U/ml penicillin G sodium, 100 U/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B, Gibco Laboratories, Grand Island, N.Y.).

Heparinized peripheral blood was collected from the prospective mother and father just before the cesarean section. Peripheral blood leukocytes were isolated by standard techniques (Sigma Diagnostics, procedure 1077). The blood was diluted 1 : 1 with culture medium and then centrifuged over Histopaque 1077 at 1000 *g* for 30 minutes. The buffy coat was aspirated and rinsed.

Separate cell suspensions of maternal peripheral blood leukocytes and decidual cells were adjusted to 10^6 cells/ml and duplicate 1 ml suspensions of each were placed in 24-well tissue culture plates. The cells were stimulated with 10 μg/ml of concanavalin A and incubated for 24 hours at 37° C. Supernatants were collected and stored at −20° C until assayed.

To evaluate the maternal peripheral lymphocyte and the decidual lymphocyte-like cell response to paternal antigen, 5×10^5 maternal peripheral blood leukocytes or decidual cells were coincubated with a similar number of irradiated (2000 rads) paternal cells for 24 hours. Unstimulated control cultures were established for both cell populations in all experiments. Culture supernatants were collected and stored at −20° C until assayed.

Supernatants were assayed for granulocyte-macrophage colony-stimulating factor and interleukin-2 with commercially available enzyme-linked immunosorbent assay kits (Genzyme, Boston). The limits of sensitivity for the interleukin-2 enzyme-linked immunosorbent assay range from 0.3 ng/ml to 20.8 ng/ml. The intraassay coefficient of variation ranges from 2% to 6% and the interassay coefficient of variation is approximately 4%. For the granulocyte-macrophage colony-stimulating factor enzyme-linked immunosorbent assay, the limits of sensitivity range from 0.008 ng/ml to 1.0 ng/ml. The intraassay coefficient of variation ranges from 2% to 8%, whereas the interassay coefficient of variation ranges from 5% to 10%.

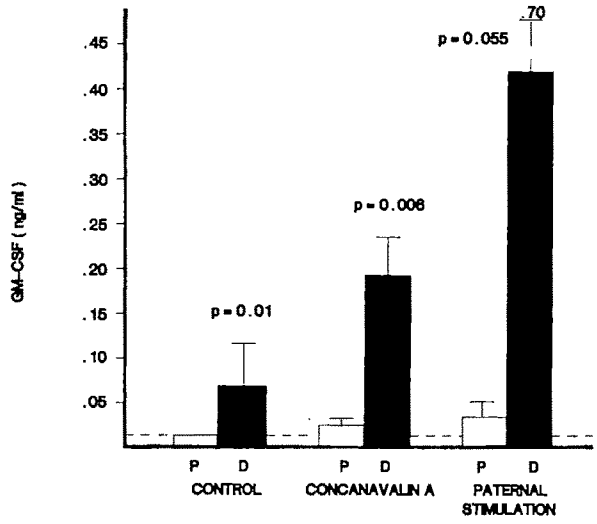


Fig. 1. Granulocyte-macrophage colony-stimulating factor production by maternal peripheral leukocytes *(P)* and decidual lymphocyte-like cells *(D)* in unstimulated, concanavalin A–stimulated, and paternal antigen-stimulated conditions ($n = 8$). The *hatched line* delineates the lower limit of sensitivity of the assay. All values are mean ± SEM.

The data were evaluated statistically with the Mann-Whitney U test.

Results

Eight term pregnancies were evaluated. Granulocyte-macrophage colony-stimulating factor production by maternal peripheral blood leukocytes and decidual lymphocyte-like cells is depicted in Fig. 1. Concanavalin A–stimulated maternal peripheral blood leukocytes produced low levels of granulocyte-macrophage colony-stimulating factor (0.018 ± 0.005 ng/ml, mean ± SEM). However, decidual lymphocyte-like cells produced much greater amounts of granulocyte-macrophage colony-stimulating factor after stimulation with concanavalin A (0.2 ± 0.059 ng/ml, $p = 0.006$). Stimulation with paternal peripheral blood leukocytes also resulted in greater production of granulocyte-macrophage colony-stimulating factor by decidual cells (0.441 ± 0.262 ng/ml, $p = 0.055$ when compared with peripheral cells stimulated with paternal peripheral blood leukocytes).

Fig. 1 also suggests that the decidual cells produce granulocyte-macrophage colony-stimulating factor constitutively at low, but significant, levels (0.073 ± 0.038 ng/ml, $p = 0.01$ when compared with unstimulated maternal peripheral cells). To evaluate whether this might reflect nonspecific stimulation caused by cell processing or culture, we performed a time course experiment with concanavalin A–stimulated and unsti-

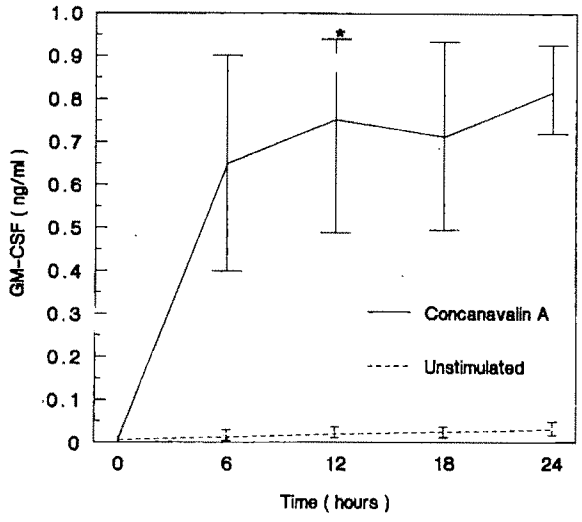


Fig. 2. Granulocyte-macrophage colony-stimulating factor production by unstimulated and concanavalin A–stimulated decidual lymphocyte-like cells over a 24-hour time period (n = 3, mean ± SE). (The standard error at the *asterisk* is 0.283.)

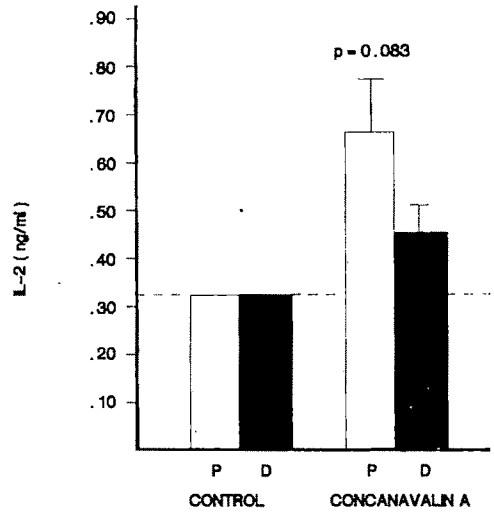


Fig. 3. Interleukin-2 production by unstimulated and concanavalin A–stimulated maternal peripheral leukocytes *(P)* and decidual lymphocyte-like cells *(D)* (n = 8). The *hatched line* delineates the lower limit of sensitivity of the assay. All values are mean ± SEM.

mulated decidual cells from three different placentas. Culture supernatants were obtained at the start of the experiment and then every 6 hours for 24 hours. The results are shown in Fig. 2. Granulocyte-macrophage colony-stimulating factor was undetectable in the cell suspensions before stimulation (time 0). However, low levels of granulocyte-macrophage colony-stimulating factor were produced by unstimulated decidual cells over 24 hours. This may represent nonspecific activation of the cells. Stimulation with concanavalin A resulted in significant production of granulocyte-macrophage colony-stimulating factor after 6 hours of incubation, indicating that the apparent nonspecific activation contributes minimally to mitogen-induced granulocyte-macrophage colony-stimulating factor production.

There was no significant difference in interleukin-2 production by maternal peripheral leukocytes and maternal decidual cells after stimulation with concanavalin A (Fig. 3). However, the P value approaches significance (p = 0.083). This lack of significance may be a result of the small sample size of the study. No interleukin-2 was detected after maternal cells were stimulated with irradiated paternal leukocytes. Irradiated paternal cells did not produce detectable amounts of interleukin-2 or granulocyte-macrophage colony-stimulating factor.

Comment

Recent observations suggest that lymphokines are important mediators of immune effector cells. Lymphokines are polypeptides secreted by activated lymphocytes with autocrine and paracrine functions to direct the immune response. They signal lymphocytes to proliferate,[6] develop cytotoxicity,[7] and produce antibody.[8] To date, more than 10 lymphokines have been described. The nature of the immune response may be dictated by the predominant lymphokine produced by stimulated lymphocytes in response to specific antigen.[9] Lymphokines also may function in a "trophic" role in certain nonlymphoid tissues.[4]

Our data indicate that immune effector cells from maternal peripheral blood and decidua differ markedly in their capacity to produce the lymphokine granulocyte-macrophage colony-stimulating factor after stimulation. Our findings also imply that assays of maternal peripheral immune cells are not reflective of the immunologic relationship between the mother and fetus. This is the first study to show that human term decidual cells produce large amounts of granulocyte-macrophage colony-stimulating factor after stimulation. Granulocyte-macrophage colony-stimulating factor is generally thought to be primarily a hematopoietic cytokine, inducing bone marrow stem cells to proliferate and differentiate.[10] However, Athanassakis et al.[11] have found that granulocyte-macrophage colony-stimulating factor produced by murine decidual cells induces proliferation of murine trophoblast.[11] This finding forms the basis of Wegmann's "immunotrophism" hypothesis.[4] According to this hypothesis, the maternal immune response to fetopaternal antigens is characterized by the secretion of granulocyte-macrophage colony-stimulating factor and other trophic cytokines. These cytokines stimulate trophoblast growth and thus maintain the fetal allograft.

Our finding that granulocyte-macrophage colony-stimulating factor is produced in large amounts by human decidual cells in response to paternal antigen supports this hypothesis. Although it is possible that the granulocyte-macrophage colony-stimulating factor produced after stimulation with paternal peripheral blood leukocytes may reflect nonspecific antigenic stimulation, we believe that this is unlikely given the large quantities produced. Whether granulocyte-macrophage colony-stimulating factor is essential to human pregnancy is unknown, but it is interesting that the heterogenous collection of decidual cells we tested maintained the capacity to produce large amounts of this lymphokine after stimulation. It seems likely that decidual granulocyte-macrophage colony-stimulating factor is of teleologic significance and it will be of scientific and academic interest to dissect the cell populations and determine the source of the granulocyte-macrophage colony-stimulating factor.

The marked differences in granulocyte-macrophage colony-stimulating factor production response between cells from the peripheral blood and the decidua suggest that the immune cells in these environments are under different regulatory control. This is consistent with our hypothesis that the maternal decidua constitutes a distinct immunologic microenvironment. In the murine immune system, cells from different lymphoid tissues produce different lymphokines.[12] An important regulatory factor is the steroid hormonal milieu found in each lymphoid tissue.[12, 13] We have recently shown that corticosteroids, dehyroepiandrosterone, and 1,25-dihydroxyvitamin D_3 at physiologic concentrations can dramatically alter lymphokine production by stimulated lymphocytes.[12, 13] Therefore markedly different lymphokine production from immune effector cells isolated from the decidua, where the cells would be influenced by the dramatic changes in the decidual steroid hormone environment, would be anticipated.

In conclusion, our data show that decidual lymphocyte-like cells differ from maternal peripheral blood leukocytes in their capacity to produce large amounts of granulocyte-macrophage colony-stimulating factor. Furthermore, we have shown a similar response to paternal antigen and this may reflect an immunotrophic maternal response to pregnancy. Further characterization of the normal lymphokine response by decidual immune effector cells and the local immunoregulatory factors that control lymphokine production will provide new insights into the normal and abnormal maternal immune responses evoked by the fetal allograft.

REFERENCES

1. Bell SC, Billington WD. Anti-fetal allo-antibody in the pregnant female. Immunol Rev 1983;75:5-30.
2. Nicholas NS, Panayi GS, Nouri AME. Human pregnancy serum inhibits interleukin-2 production. Clin Exp Immunol 1984;58:587-95.
3. Lala PK, Chatterjee-Hasrouni S, Kearns M, Montgomery B, Colavincenzo V. Immunobiology of the maternal-fetal interface. Immunol Rev 1983;75:87-116.
4. Wegmann TG. Placental immunotrophism: the idea and the evidence. In: Chaouat G, ed. The immunology of the fetus. Boca Raton, Florida: CRC Press, Inc, 1990:179.
5. Delvin EE, Arabian A, Glorieux FH, Mamer OA. In vitro metabolism of 25-hydroxycholecalciferol by isolated cells from human decidua. J Clin Endocrinol Metabol 1985; 60:880-5.
6. Mosmann TR, Coffman RL. Th1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Ann Rev Immunol 1989;7:145-73.
7. Farrar JJ, Benjamin WR, Hilfiker ML, Howard M, Farrar WL, Fuller-Farrar J. The biochemistry, biology, and role of interleukin 2 in the induction of cytotoxic T cell and antibody-forming B cell responses. Immunol Rev 1982; 63:129-66.
8. Coffman RL, Seymour BW, Lebman DA, et al. The role of helper T cell products in mouse B cell differentiation and isotype regulation. Immunol Rev 1988;102:5-28.
9. Janeway CA, Carding S, Jones B, et al. CD4+ T cells: specificity and function. Immunol Rev 1988;101:39-79.
10. Tomomonga M, Golde DW, Gasson JC. Biosynthetic (recombinant) human granulocyte-macrophage colony-stimulating factor: effect on normal bone marrow and leukemia cell lines. Blood 1986;67:31-6.
11. Athanassakis I, Bleackley RC, Paetkau V, Guilbert L, Barr PJ, Wegmann TG. The immunostimulatory effect of T cells and T cell lymphokines on murine fetally-drived placental cells. J Immunol 1987;138:37-44.
12. Daynes RA, Araneo BA, Dowell TA, Huang K, Dudley DJ. Regulation of murine lymphokine production in vivo: regulatory influences over T helper cell function. J Exp Med 1990;171:979-96.
13. Daynes RA, Dudley DJ, Araneo BA. Regulation of murine lymphokine production in vivo: II. Dehydroepiandrosterone is a natural enhancer of IL-2 synthesis by helper T cells. Eur J Immunol 1990;20:793-802.

The effect of maternal hyperoxia on behavioral activity in growth-retarded human fetuses

Robert Gagnon, MD, Cora Hunse, RN, and Shubnum Vijan, BSc
London, Ontario, Canada

Thirteen pregnant women who subsequently were delivered of infants with birth weights less than the 3rd percentile were studied for examination of fetal heart rate and fetal activity patterns during maternal administration of oxygen at a concentration of 50% or room air for 2 hours. None of the fetuses was acidotic at birth. Maternal transcutaneous P_{O_2} levels increased from 79 ± 3 mm Hg to 158 ± 10 mm Hg for the 2 hours of observation. The results indicated that maternal hyperoxia produced sustained fetal breathing activity that was almost 100% higher than that in room air (analysis of variance, $p = 0.024$). Gross fetal body movements, fetal heart rate accelerations, and fetal heart rate variability increased significantly with increasing observation time (analysis of variance, $p < 0.01$), but were not significantly altered by maternal hyperoxia or room air. We conclude that despite significant change in fetal breathing activity, ultrasonographic observation of fetal behavioral activity during maternal hyperoxia could not be used to differentiate severely growth-retarded from normally grown human fetuses. We speculate that altered fetal heart rate and fetal body movement patterns usually associated with intrauterine growth retardation might be related to altered development of the fetal central nervous system and are not reversible during prolonged maternal administration of oxygen. (Am J Obstet Gynecol 1990;163:1894-9.)

Key words: Fetal behavior, intrauterine growth retardation, maternal hyperoxia

In pregnancies complicated with severe intrauterine growth retardation (IUGR) [small-for-gestational-age (SGA) fetuses] fetal heart rate (FHR) variability, the number of FHR accelerations, fetal breathing, and gross fetal body movements are often reduced.[1,2] It is usually believed that changes in behavioral activity in SGA fetuses are a result of chronic hypoxemia subsequent to placental insufficiency.[3] Maternal administration of oxygen has been suggested as a therapeutic approach to hypoxic fetuses,[4] and as a diagnostic test to discriminate the growth-retarded (SGA) from the normally grown [appropriate-for-gestational-age (AGA)] fetuses.[5]

The aims of this study were to determine the effects of prolonged maternal hyperoxia on FHR, FHR variability, and movement patterns in a group of SGA fetuses from 30 weeks' gestation to term.

Patients and methods

Patients. Informed consent was obtained from 21 pregnant women (investigation protocol approved by the Ethics Review Board for Research Involving Human Subjects) with singleton pregnancies between 30 and 40 weeks' gestation with clinical or ultrasonographic findings suspicious for IUGR (SGA fetus). All patients either had reliable menstrual dates or underwent ultrasonographic examination during the second trimester. Usher and McLean[6] charts were used to classify the birth weights at sea level of the fetuses studied. Growth retardation was diagnosed when birth weight was less than the 3rd percentile for gestational age. The 3rd percentile at sea level is similar to the 10th percentile for birth weights of the Denver standards[7] and is used as a cutoff point to diagnose IUGR in our center. A total of seven (33%) of the 21 participants with fetuses thought to be SGA had normal term deliveries with normal birth weights and were analyzed separately from SGA fetuses. All other participants were delivered of infants with a birth weight less than the 3rd percentile. There was no congenital anomaly or neonatal death. Umbilical cord arterial and venous blood gas values were obtained in all fetuses. Table I illustrates the characteristics and umbilical cord gas values of the SGA fetuses studied and Table II shows the primary maternal risk factors associated with IUGR. Three (75%) of four symmetrical IUGR fetuses were clinically detected before 33 weeks' gestation (early onset) and eight (88.9%) of nine asymmetrical IUGR fetuses were clinically detected at ≥33 weeks' gestation (late onset). Five (38.5%) women were delivered of infants by cesarean section and eight (61.5%) had vaginal delivery.

Experimental design. The women received a stan-

From the Departments of Obstetrics and Gynecology, Lawson Research Institute, St. Joseph's Health Centre, University of Western Ontario.
Supported by the Physicians' Services Incorporated Foundation and the Canadian Medical Research Council. R. G. is a Medical Research Council of Canada Scholar.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Dr. Robert Gagnon, Department of Obstetrics and Gynecology, St. Joseph's Health Centre, 268 Grosvenor St., London, Ontario, Canada, N6A 4V2.
6/6/24754

dardized 800 kcal breakfast at 8 AM or lunch at noon on two consecutive half days. All studies began 30 minutes later and were conducted in a quiet room with patients either sitting upright in bed or resting in a lateral recumbent position. The women were asked not to smoke for at least 4 hours before the studies were conducted.

Women were studied continuously for 3 1/2 hours on each of 2 half days. During the first episode of low FHR variability (mean minute range of <32 msec for >5 minutes duration) of each consecutive half day, oxygen was administered for 2 hours through a ventimask with either a 50% oxygen or 21% room air concentration of inspired oxygen. This technique allowed us to synchronize all fetuses in a state of quietness for the same length of observation (10 minutes) just before both oxygen and room air were administered. The choice of whether to administer oxygen or room air the first or second half day was made from random number tables. Maternal Po_2 was continuously measured with a transcutaneous oxygen monitor (Oxymonitor, Litton Medical Electronics, Boston, Mass.). All women were then observed for 2 hours during the time oxygen and room air were administered.

FHR and fetal activity measurements. FHR was recorded with an external monitor with a compact transducer (model 8040A, Hewlett-Packard GMBH, Boeblinger, Federal Republic of Germany) and analyzed on-line by a Sage II microprocessor (Sage Technology, Reno, Nev.) as previously described.[2] During analysis, a baseline was fitted to the heart rate record and accelerations of ≥10 beats/min for ≥15 seconds above the baseline were recognized automatically.

For each 10-minute interval, the basal FHR (in beats per minute), the mean minute range (computer-derived index of FHR variability in milliseconds), and the total gained beats (integrated area of FHR accelerations) were calculated.

Fetal breathing movements and gross fetal body movements were measured with a real-time ultrasonographic scanner (model 2130, Advanced Diagnostic Research Corp., Tempe, Ariz.) and analyzed on-line as previously described.[8] The 3.5 MHz transducer was positioned over the maternal abdomen to permit continuous observation of a longitudinal cross section of fetal chest and abdominal wall echoes.[8]

Data analysis. The basal FHR, the number of FHR accelerations of ≥10 beats/min for ≥15 seconds above the baseline, the mean minute range, and the total gained beats were determined for successive 10-minute intervals during the 68 hours of total FHR recordings. All results are presented as means ± SEMs for successive 10-minute intervals before and during administration of oxygen or room air. Portions of records that could not be analyzed because of FHR signal loss of >30% or patient interruption were not included. These portions made up 6.8% of the total study time. One woman was delivered of an infant before the second half day of the study and was excluded for the purpose of analysis.

Measurements of the percentage of time spent making gross fetal body movements and the percentage of time spent making breathing movements were calculated for each 10-minute interval. Portions of movement records that could not be analyzed because of technical problems or patient interruption made up 6.0% of the total time involved.

Statistical significance between groups after administration of oxygen or room air was determined by a two-factor analysis of variance with repeated measures, comparing overall means and each of the 10-minute intervals with biomedical data processing 5V statistical software.[9]

Table I. Patient characteristics

	Mean ± SEM	*Range*
Gestational age at test (wk)	34.0 ± 1.6	29.7-39
Gestational age at delivery (wk)	35.7 ± 1.7	31-41
Birth weight (gm)	1789 ± 264	440-2750
Placental weight (gm)	328 ± 22	154-460
Symmetrical*	4	—
Asymmetrical† (No.)	9	—
5-min with Apgar score <7 (No.)	1	—
Po_2 (mm Hg)		
Artery	14.9 ± 1.9	7.1-20.4
Vein	26.8 ± 2.8	15.9-39.2
Pco_2 (mm Hg)		
Artery	57.5 ± 3.6	39.4-69.5
Vein	44.8 ± 2.8	22.0-55.1
pH		
Artery	7.26 ± 0.02	7.18-7.35
Vein	7.33 ± 0.02	7.26-7.41

*Birth weight, head circumference, length <3rd percentile.
†Birth weight <3rd percentile, and head circumference plus length >3rd percentile.

Table II. Primary maternal risk factors

	No.	*%*
Pregnancy-induced hypertension	6	46.2
Smoking >20 cigarettes/day	5	38.5
Chronic hypertension	1	7.7
Previous IUGR	1	7.7

Results

Fetal breathing and gross fetal body movements. The mean time from the onset of the study until an

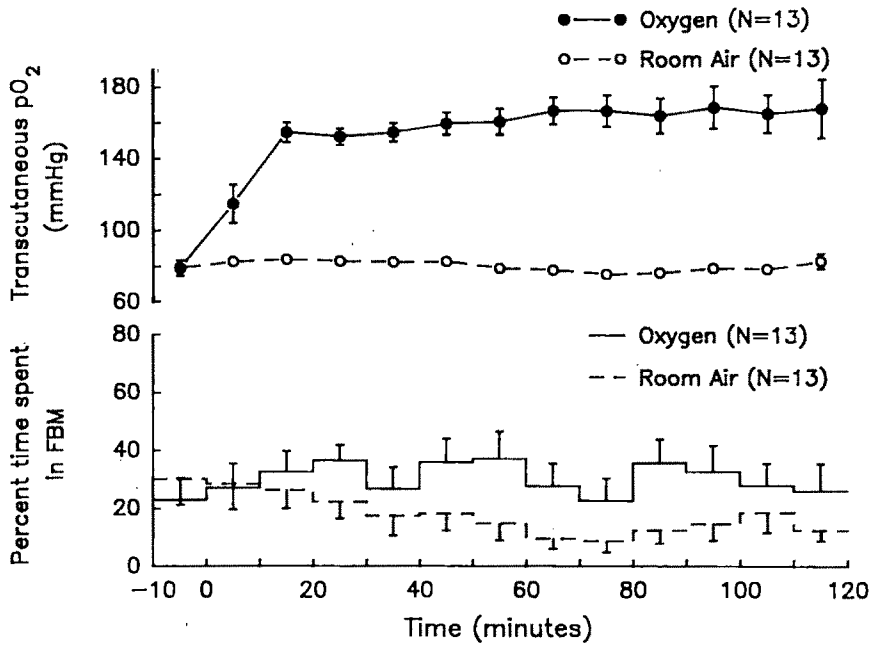


Fig. 1. Mean maternal transcutaneous Po_2 (mm Hg) ± SEM was plotted in 10-minute intervals before and after maternal administration of oxygen *(solid lines)* or room air *(dashed lines)* **(top).** Mean incidence of fetal breathing movements *(bottom)* was also plotted on same time scale. *Horizontal bar* indicates time during which oxygen or room air was administered.

episode of low FHR variability occurred was 21 ± 4 minutes before administration of oxygen compared with 25 ± 3 minutes before room air. During maternal hyperoxia the mean transcutaneous oxygen increased from 79 ± 3 mm Hg to a maximum of 158 ± 10 mm Hg at time 0 plus 15 minutes and remained above that of room air during the 2 hours of observation (analysis of variance, $p < 0.001$, Fig. 1, top).

Mean fetal breathing incidence was similar during the 10 minutes before administration of oxygen (23.0% ± 6.7%) or room air (30.1% ± 9.1%). During the 2 hours oxygen was administered, the mean incidence of fetal breathing movements was 33.5% ± 4.5%, which was significantly higher than that of 18.4% ± 3.3% during room air (analysis of variance, $p = 0.024$). However, there was no significant effect of time on the incidence of fetal breathing movements during maternal hyperoxia, suggesting that fetal breathing movements were sustained during hyperoxia but not markedly increased above preoxygen levels (Fig. 1, bottom). In contrast, fetal breathing movements decreased progressively to 12.3% ± 5.5% between 110 and 120 minutes after administration of room air was started (Fig. 1, bottom).

The mean incidence of gross fetal body movements per 10 minutes increased significantly from 3.9% ± 1.3% before administration of oxygen to a maximum of 11.6% ± 2.5% 40 minutes after the onset of maternal administration of oxygen (analysis of variance, $p = 0.002$; Fig. 2, top). A similar increase in the mean incidence of gross fetal body movements was found during administration of room air and there was no significant difference between the two groups (oxygen and room air, respectively, Fig. 2, top).

FHR accelerations, FHR variability, and basal FHR. The mean total gained beats per 10 minutes, which was 4.2 ± 2.1 before oxygen was administered, increased to a maximum of 21.5 ± 6.1 between 30 and 40 minutes after the onset of maternal hyperoxia (analysis of variance, $p = 0.01$; Fig. 2, bottom). Similarly, the mean number of FHR accelerations per 10 minutes increased from 0.7 ± 0.2 before administration of oxygen to a maximum of 2.4 ± 0.6 accelerations between 30 and 40 minutes of maternal hyperoxia (analysis of variance, $p = 0.004$). The mean 10-minute mean minute range, which was 29.1 ± 2.6 msec before administration of oxygen, increased progressively to 46.9 ± 6.2 msec at 40 minutes of maternal hyperoxia (analysis of variance, $p < 0.001$). No significant difference was found between room air and oxygen in FHR variability and FHR accelerations. The mean basal FHR was 136 ± 3 beats/min before oxygen and did not change during the 2 hours of maternal administration of oxygen or room air.

Relationship between fetal behavioral activity and FHR patterns. Fig. 3 summarizes characteristics of FHR and fetal activity patterns during the 2 hours in which SGA fetuses received oxygen or room air. During

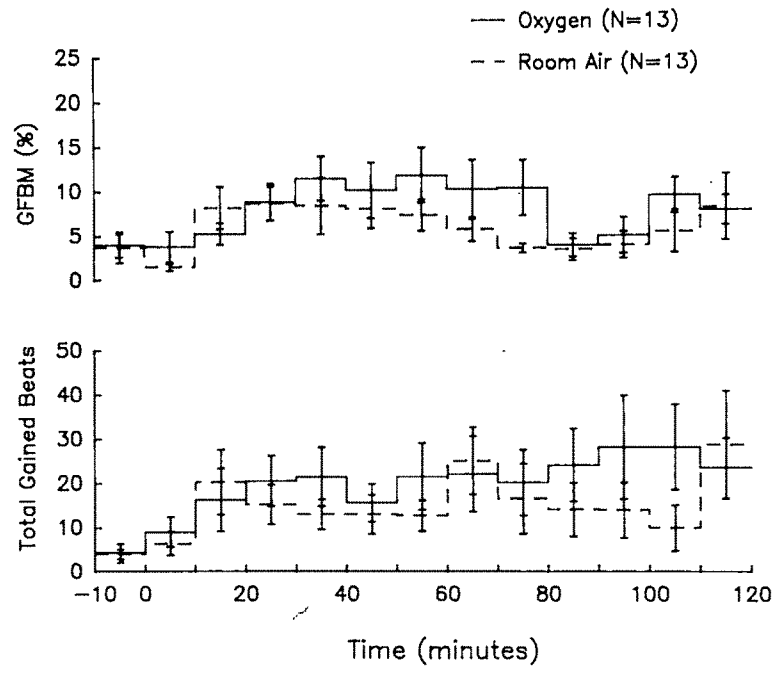


Fig. 2. Mean incidence of gross fetal body movements *(GFBM)* was plotted in 10-minute intervals before and after maternal administration of oxygen *(solid lines)* or room air *(dashed lines)* (top). Mean total gained beats (bottom) was also plotted on same time scale. *Horizontal bar* indicates time during which oxygen or room air was administered.

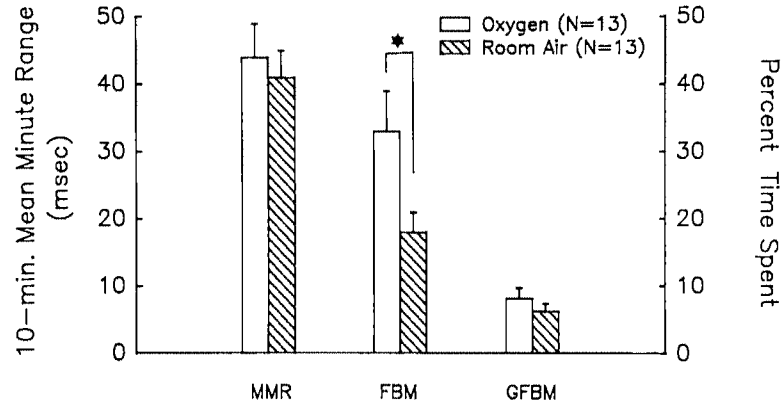


Fig. 3. Mean incidence of fetal breathing movements *(FBM)*, mean incidence of gross fetal body movements *(GFBM)*, and mean 10-minute mean minute range *(MMR)* were plotted during 2 hours of maternal administration of oxygen or room air ($*p < 0.05$).

the 2 hours of maternal hyperoxia, the fetuses made breathing movements 33.5% ± 4.5%, which was significantly higher than during the time room air was administered (18.4% ± 3.3%) (analysis of variance, $p = 0.024$). However, it was important that the incidence of gross fetal body movements and the 10-minute mean minute range were not significantly different between oxygen and room air (room air values: 6.2% ± 1.2% for gross fetal body movements and 41.5 ± 4.1 msec for 10-minute mean minute range value, respectively, Fig. 3).

In the seven fetuses who were subsequently born as AGA infants, the mean fetal breathing movements incidence was 36.4% ± 5.4% during the 2 hours room air was administered, which was not significantly different from the 38.3% ± 4.5% during maternal hyperoxia. Similarly, the mean incidence of gross fetal body movements, the mean 10-minute range, and the mean total gained beats were not significantly altered during maternal administration of oxygen (9.1% ± 1.5% for gross fetal body movements, 50.5 ± 2.3 msec for 10-minute mean minute range value, and 21.0 ±

2.3 for total gained beats) compared with room air (10.1% ± 1.7%, 54.5 ± 2.9 msec, and 22.4 ± 3.1, respectively).

Comment

The data show that in a group of severely growth-retarded fetuses, maternal administration of oxygen (inspired oxygen 50%) was associated with sustained fetal breathing movements compared with room air. In contrast, fetal body movements and computer-based indexes of FHR accelerations and FHR variability were not significantly altered under similar experimental conditions. In fetuses that were subsequently delivered as AGA infants, the incidence of fetal breathing movements, the incidence of gross fetal body movements, and FHR variability did not change significantly during maternal hyperoxia.

The periodicity in rest-activity cycles of the human fetus is well accepted and has provided the physiologic basis for fetal assessment.[10] Our data and others[1, 2] indicated that such cycles are present in SGA fetuses between 30 and 40 weeks' gestation. When observations of fetal activity were started during a period of rest, with increasing observation time, SGA fetuses became more active, experienced more FHR accelerations with increased FHR variability reaching a peak between 30 and 40 minutes (Fig. 2). We recently described the same patterns of behavioral activity with the use of similar experimental design in a group of healthy term AGA fetuses.[11] However, maternal hyperoxia sufficient to double maternal transcutaneous P_{O_2} did not alter fetal rest-activity cycles in growth-retarded fetuses.

Ruedrich et al.[12] recently reported an increase in fetal body movements in IUGR fetuses during maternal hyperoxia reaching a peak 30 minutes after oxygen was discontinued. Because there was no control group without oxygen in the experiment, it is possible that the increase in fetal body movements described in the study was a result of the normal periodicity of rest-activity cycles rather than oxygen alone.

A significant portion (50%) of the SGA fetuses being tested and delivered during the present study were premature (<37 completed weeks' gestation), as expected with severe IUGR. The organization of biophysical activities into clearly differentiated behavioral states as defined by Nijhuis et al.[13] usually occurs after 36 weeks' gestation in AGA fetuses. In contrast, the same group of investigators reported the absence of states differentiation in up to 80% of SGA fetuses at 40 weeks' gestation.[14] Although prematurity at the time of the studies may account for the differences between our observations and others,[12] it is very unlikely that the establishment of behavioral states would have occurred in SGA fetuses even in term and that results would have been different at later gestational age. Moreover, incidence of fetal breathing movement is not used as a state variable[13] because in contrast to the fetal lamb, it is present in both quiet and active sleep states in the human fetus.[13]

Although fetal body movements and FHR accelerations usually occur together, there is some evidence that the control of fetal breathing movements could be dissociated from other biophysical variables used to assess fetal health and behavioral activity. In the fetal lamb, prolonged hypoxemia suppresses fetal breathing activity without significant change in electrocortical activity.[15] Fetal breathing activity is suppressed by maternal administration of ethanol[16] and stimulated by infusion of glucose[17] in the healthy term human fetus. In both experimental conditions, fetal body movement and FHR patterns are not altered.[16-17] In this study, as in others,[18] the incidence of fetal breathing movements in SGA fetuses was significantly higher during maternal hyperoxia than that in room air. It is not clear by which mechanisms maternal administration of oxygen can produce sustained breathing activity. It has been reported in work by Boddy et al.[19] in the fetal lamb, that during maternal hyperoxia there is an increase in fetal P_{CO_2} without change in maternal P_{CO_2} only if the fetus was previously hypoxemic. Hypercapnia is known to be a potent stimulus of fetal breathing activity and could be responsible for the sustained breathing activity. However, this mechanism is speculative and remains to be confirmed.

It has been shown that fetuses with severe IUGR have a reduction in long-term FHR variability, FHR accelerations, and fetal movements.[1-3] Maternal hyperoxia did not have any significant effect on these biophysical variables in the present study. This suggested that altered behavioral activity associated with IUGR might be related to an abnormal development of the fetal central nervous system and is not reversible during prolonged maternal administration of oxygen.

Our observation that maternal hyperoxia is associated with sustained fetal breathing incidence but without change in body movements and FHR accelerations in SGA fetuses during the third trimester, does not support the concept that ultrasonographic observation of fetal behavioral activity during maternal hyperoxia could be used to differentiate SGA from AGA fetuses as recently suggested.[12] Moreover, it is possible to speculate that chronic maternal administration of oxygen used as fetal therapy for SGA fetuses[4] may not improve fetal outcome at a time IUGR is clinically or ultrasonographically diagnosed. These important questions remain to be answered with appropriate clinical trials before suggesting the use of maternal hyperoxia for intrauterine evaluation or treatment.

We thank Mrs. Maria De Sousa for her excellent technical assistance.

REFERENCES

1. Henson G, Dawes GS, Redman CWG. Characterization of the reduced heart rate variation in growth-retarded fetuses. Br J Obstet Gynaecol 1984;94:751-5.
2. Gagnon R, Hunse C, Bocking AD. Fetal heart rate patterns in the small-for-gestational-age human fetus. AM J OBSTET GYNECOL 1989;161:779-84.
3. Bekedam DJ, Visser GHA, Mulder EJH, et al. Heart rate variation and movement incidence in growth-retarded fetuses: the significance of antenatal late heart rate decelerations. AM J OBSTET GYNECOL 1987;157:126-33.
4. Nicolaides KH, Campbells, Bradley RJ, et al. Maternal oxygen therapy for intrauterine growth retardation. Lancet 1986;1:942-7.
5. Arduini D, Rizzo G, Mancuso S, et al. Short-term effects of maternal oxygen administration on blood flow velocity waveforms in healthy and growth-retarded fetuses. AM J OBSTET GYNECOL 1988;159:1077-80.
6. Usher R, McLean F. Intrauterine growth of live-born caucasian infants at sea level. J Pediatr 1969;74:901.
7. Lubchenco LO, Hansman C, Boyd E. Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 42 weeks. Pediatrics 1966;37:403.
8. Patrick J, Campbell K, Carmichael L, et al. A definition of human fetal apnea and the distribution of fetal apneic intervals during the last ten weeks of pregnancy. AM J OBSTET GYNECOL 1980;136:471.
9. Dixon WJ. BMDP statistical software manual. Berkeley, California: University of California Press, 1988, vol 1.
10. Patrick J. The physiological basis for fetal assessment. Semin Perinat 1989;13:403-8.
11. Gagnon R, Hunse C, Foreman J. Human fetal behavioral states after vibratory stimulation. AM J OBSTET GYNECOL 1989;1470-6.
12. Ruedrich DA, Devoe LD, Searle N. Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation. AM J OBSTET GYNECOL 1989;161:188-92.
13. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioural states in the human fetus? Early Hum Dev 1982;6:177-95.
14. Van Vliet MAT, Martin CB Jr, Nijhuis JG, Prechtl HFR. Behavioural states in growth-retarded human fetuses. Early Hum Dev 1985;12:183-97.
15. Bocking AD, Gagnon R, Milne KM, et al. Behavioral activity during prolonged hypoxemia in fetal sheep. J Appl Physiol 1988;65:2420-6.
16. Patrick J, Richardson B, Hasen G, et al. Effects of maternal ethanol infusion on fetal cardiovascular and brain activity in lambs. AM J OBSTET GYNECOL 1985;151:859-67.
17. Natale R, Richardson B, Patrick J. Effect of intravenous glucose infusion on human fetal breathing activity. Obstet Gynecol 1982;59:320-4.
18. Dornan JC, Ritchie JWK. Fetal breathing movements and maternal hyperoxia in the growth retarded fetus. Br J Obstet Gynaecol 1983;90:210-3.
19. Boddy K, Dawes GS, Fisher R, et al. Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. J Physiol (London) 1974;243:599-618.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Observations on the cause of oligohydramnios in prolonged pregnancy

Kenneth J. Trimmer, MD, Kenneth J. Leveno, MD, Mark T. Peters, MD, and Mary Anne Kelly, RN

Dallas, Texas

There is increasing evidence that implicates reduced amnionic fluid volume as a major determinant of fetal risk in prolonged pregnancy. We sought to determine whether reduced fetal urine production might be associated with oligohydramnios in pregnancies that reach 42 weeks or more. Ultrasonographic measurements of the fetal bladder were obtained every 2 to 5 minutes for 1 hour in 38 gestations verified to be at least 42 weeks. Oligohydramnios was present in eight of the prolonged pregnancies. Similar measurements were performed in 15 normal pregnancies delivered by elective repeat cesarean section between 38 and 40 weeks' gestation. Hourly fetal urine production rates were calculated with sequential bladder volume measurements. The result of this investigation suggest that diminished fetal urine production is associated with oligohydramnios in prolonged pregnancy. The mechanism by which fetal urine production is reduced in prolonged pregnancy remains unknown. A likely possibility is reduced fetal swallowing because of already diminished amnionic fluid volume, the latter a result of placental senescence. (Am J Obstet Gynecol 1990;163:1900-3.)

Key words: Postterm pregnancy, fetal urine production, oligohydramnios, ultrasonography

Perinatal morbidity is significantly increased in postterm pregnancies and oligohydramnios appears to be an important factor.[1] The inability to accurately measure diminished amnionic fluid volume has led to the adoption of ultrasonographic semiquantitative estimations of volume such as the four-quadrant method[2] or the measurement of the deepest vertical pocket.[3] Because fetal urine is a major source of amnionic fluid, measurement of hourly fetal urine production rates may provide information on amnionic fluid dynamics in prolonged pregnancies. Indeed, realtime ultrasonography has been used to quantitate hourly fetal urine production rates in normal term pregnancies[4] and in pregnancies complicated by intrauterine growth retardation.[5] The purpose of this investigation was to measure and compare fetal urine production rates in normal term pregnancies, postterm pregnancies with normal amnionic fluid volume, and those with oligohydramnios.

Material and methods

Women with singleton pregnancies verified to be 42 to 43 weeks were recruited from the Obstetrical Complications Clinics at Parkland Memorial Hospital. Labor was induced the next morning. Gestational ages were calculated from a calendar of recorded last menses and two or more of the following: (1) first trimester examination, (2) fundal height measurements between 20 and 30 weeks' gestation, (3) DeLee-Hillis fetoscopic fetal heart tones detected between 18 and 20 weeks' gestation, and (4) ultrasonography performed before 24 weeks' gestation. In addition, normal singleton pregnancies at 38 to 40 weeks' gestation scheduled the next morning for repeat cesarean section were selected to serve as controls.

All pregnancies received ultrasonographic examination with a 3.5 MHz linear transducer (Ultramark 4, Advanced Technology Laboratories, Bothell, Wash.). Biparietal diameters, femur lengths, and abdominal perimeters were obtained in each pregnancy. Oligohydramnios was diagnosed when the maximum vertical dimension of the largest amnionic fluid pocket measured $\leq$ 1 cm.[3]

Hourly fetal urine production rates were calculated once in each pregnancy and within 24 hours of delivery or labor induction. The method used was identical to that previously described by Rabinowitz, Peters, Vyas, et al.[4] Briefly, Rabinowitz and colleagues used real-time ultrasonography to obtain measurements of the fetal bladder every 2 to 5 minutes for 1 hour. The greatest longitudinal bladder dimension (sector a) was measured as shown in Fig. 1. The transducer was then rotated 90° and the bladder scanned cephalad and caudad as shown in Fig. 2. The greatest trans-

From the Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Kenneth J. Trimmer, MD, and Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9032.
6/6/24745

verse distance (sector b) and anterior-posterior dimension (sector c) of the fetal bladder were then measured. Bladder volumes were calculated from these three sector measurements with the formula for an ovoid sphere: $4/3\ \pi \times a/2 \times b/2 \times c/2$, where a = longitudinal bladder, b = greatest transverse bladder distance, and c = anterior-posterior dimension of the fetal bladder. These bladder volumes were plotted versus time and then simple linear regression analysis was used to compute hourly fetal urine production rates on the basis of the filling phase of the first micturition cycle.

Obstetric and neonatal outcomes were obtained from the hospital records. In addition, neonatal examinations were performed by the author (K. J. T.) to specifically note physical signs of postmaturity. The infant was classified as postmature if it fulfilled all criteria of any stage of Clifford's descriptions for postmaturity.[6]

Statistical analysis was performed on a VAX 8800 computer with an interactive statistical package (UTSTAT), University of Texas Southwestern Medical Center at Dallas) with the use of Newman-Keuls multiple comparison analysis, the Student t test, and χ^2 where appropriate. Significance was considered when probabilities were < 0.05.

Results

A total of 38 pregnancies diagnosed to be 42 weeks in duration were studied. Oligohydramnios was diagnosed in eight of these pregnancies. Another 15 pregnancies delivered by elective cesarean section made up the control group. Maternal characteristics that included age, parity, and race did not differ significantly among the pregnancies. Approximately 80% of the women were Hispanic or African-American. All infants were live born and there were no neonatal deaths. One infant of a 42-week pregnancy without oligohydramnios was diagnosed to have Hirschprung's disease. Transient tachypnea of the newborn developed in another infant of a 42-week pregnancy with normal amnionic fluid volume.

Shown in Table I are selected pregnancy outcomes in postterm pregnancies with and without oligohydramnios compared with normal term gestations delivered by repeat cesarean section. Mean birth weights, although greater in those pregnancies that reached 42 weeks, were not significantly different between the study groups. Newborn condition as reflected by umbilical artery blood pH results are also shown in Table I and did not differ significantly among the study groups. Approximately 25% of the postterm pregnancies with or without oligohydramnios were delivered by cesarean section for either dystocia or fetal distress in labor. Meconium was found in the amnionic fluid of 13% ($n = 2$) of pregnancies delivered by elective

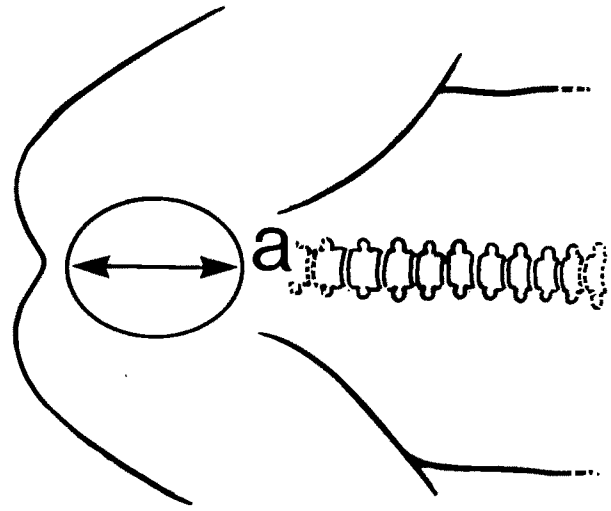


Fig. 1. Greatest longitudinal dimension shown as *sector a.*

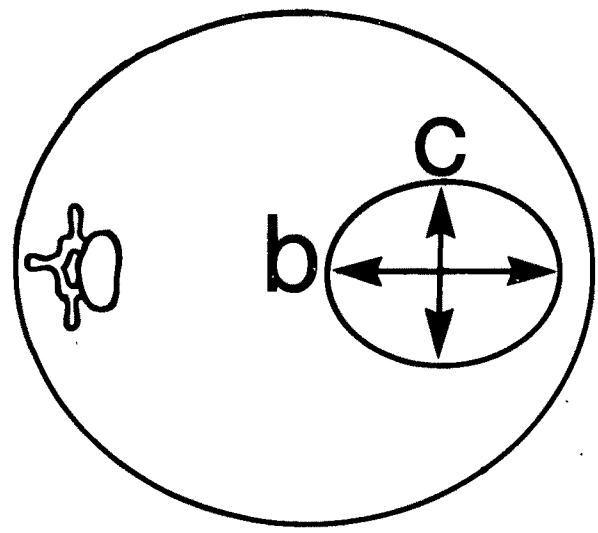


Fig. 2. Transducer is rotated 90° to measure greatest transverse and anterior-posterior bladder dimensions, *sectors b and c,* respectively.

cesarean section and in 13% ($n = 4$) of the postterm pregnancies with normal amnionic fluid volume. In contrast, meconium-stained amnionic fluid was present in 38% ($n = 3$) of the postterm pregnancies with oligohydramnios. This difference was significant ($p < 0.001$). Similarly, infants with features of the postmaturity syndrome were more common in 42-week pregnancies with oligohydramnios. Specifically, 88% ($n = 7$) of these latter pregnancies resulted in postmature infants compared with 37% ($n = 12$) when normal amnionic fluid volumes were present ($p < 0.001$).

The hourly fetal urine production rates, mean maximum bladder volumes (maximum fetal bladder vol-

Table I. Selected outcomes in postterm pregnancies with and without oligohydramnios compared with normal term gestations

	Postterm, normal amnionic fluid volume	*Postterm, oligohydramnios*	*Repeat cesarean section at term*	*p Value*
No. of pregnancies	30	8	15	—
Mean (SD) birth weight (gm)	3459 (386)	3892 (557)	3250 (542)	NS
Mean (SD) umbilical artery blood pH	7.27 (0.09)	7.27 (0.04)	7.29 (0.06)	NS
Cesarean section	8 (27%)	2 (25%)	—	NS
Meconium in amniónic fluid	4 (13%)	3 (38%)	2 (13%)	<0.001
Postmature infant*	12 (37%)	7 (88%)	0	<0.001

*According to Clifford.[6]

Table II. Fetal urodynamics in postterm pregnancies with and without oligohydramnios compared with normal term gestations

	Postterm, normal amnionic fluid volume	*Postterm, oligohydramnios*	*Repeat cesarean section at term*	*p Value*
Mean (SD) fetal urine production (ml/hr)	57 (12)	24 (3)	56 (12)	<0.001
Number of bladder cycles	29	6	17	—
Mean (SD) maximum bladder volume (ml)	38 (12)	31 (10)	33 (7)	NS
Mean (SD) bladder filling time (minutes)	25 (8)	49 (10)	23 (5)	<0.001

ume immediately before micturition), and mean cycle lengths (time for empty fetal bladder to fill and empty) for the 53 pregnancies in this study are shown in Table II. The mean maximum bladder volume was 36 ml and did not vary significantly among the study groups. Fetal micturition, observed on 93 occasions, was generally completed in <30 seconds. Postmicturition bladder volumes <25% of the maximum bladder volume were considered complete emptying and occurred in 87% of the fetuses. The mean hourly fetal urine production rates observed in the postterm pregnancies with normal amnionic fluid volumes was identical to those measured in normal term gestations. However, a precipitous reduction (50%) in fetal urine production was observed in those postterm pregnancies complicated by oligohydramnios. Specifically, hourly fetal urine production rates decreased from 56 ml/hour to 24 ml/hour ($p < 0.001$) when oligohydramnios was present. This diminished fetal urine production rate correlated directly with a prolonged fetal bladder filling cycle (the time to fill and empty the fetal bladder). The mean duration of the 46 cycles observed in the term and postterm pregnancies with normal amnionic fluid was 24 minutes compared with 49 minutes observed in six cycles in the postterm pregnancies with oligohydramnios ($p < 0.001$).

Comment

Oligohydramnios is increasingly emphasized as a hallmark of perinatal pathology in a large number of pregnancy complications,[7-9] and is particularly relevant in prolonged pregnancy because the volume of amnionic fluid diminishes as gestation reaches term and beyond. In this study, the hourly fetal urine production rate was significantly less in the postterm pregnancy complicated by oligohydramnios, whereas fetal urine production in the postterm pregnancy with normal amnionic fluid was no different than that in normal pregnancy at term.

Although amnionic fluid dynamics are not completely understood in human beings, it is known that fetal urination and swallowing play prominent roles in

the regulation of fluid volume. However, other regulatory factors are also considered important in the maintenance of amnionic fluid volume. For example, Gilbert and Brace[10] proposed an *intramembranous pathway* in which amnionic fluid is absorbed by the fetal blood perfusing the membranes and surface of the placenta. This was hypothesized to explain their findings that infusion of warmed distilled water into the amnionic fluid of ewes resulted in a decrease in fetal serum osmolality. In a more recent report, Brace studied ovine fetal blood volume, urine production, swallowing, and amnionic fluid volume in response to fetal intravascular infusion of isotonic saline solution.[11] He observed a large increase in fetal urine production concomitant with doubling of fetal swallowing. Very little of the intravenously administered saline solution remained in the fetus (1% to 2%), approximately 30% remained in the amnionic fluid, and the bulk of the infused saline solution presumably was transferred into the mother. Because fetal-maternal osmolalities did not change, Brace postulated that shifts of fetal fluids were governed by unknown placental mechanism(s) more complex than simple osmotic diffusion. These findings may have significance in the interpretation of our results now reported in prolonged human pregnancy. Specifically, our finding of markedly diminished fetal urine production when oligohydramnios complicates postterm pregnancy could conceivably be a consequence of diminished swallowing because of prior reduction of amnionic fluid volume, the latter a result of the placental factors hypothesized by Brace. That is, advanced placental maturation or senescence associated with postmaturity may be the placental factor that permits loss or transfer of amnionic fluid, presumably, into the mother.

In conclusion, prolonged pregnancy complicated by oligohydramnios is associated with marked reduction in fetal urine production. We hypothesize that this decrease in fetal urine flow is a consequence, as opposed to a primary event, of already reduced amnionic fluid volume that results from placental senescence because of prolonged gestation.

REFERENCES

1. Leveno KJ, Quirk JG, Cunningham FG, et al. Prolonged pregnancy. I. Observations concerning the causes of fetal distress. AM J OBSTET GYNECOL 1984;150:465-73.
2. Phelan JP, Smith CV, Broussard P, Small M. Amnionic fluid volume assessment with the four-quadrant technique at 36-42 weeks' gestation. J Reprod Med 1987;32:540-2.
3. Manning FA, Hill LM, Platt LD. Qualitative amnionic fluid volume determination by ultrasound: antepartum detection of intrauterine growth retardation. AM J OBSTET GYNECOL 1981;139:254-8.
4. Rabinowitz R, Peters MT, Vyas S, Campbell S, Nicolaides KH. Measurement of fetal urine production in normal pregnancy by real-time ultrasonography. AM J OBSTET GYNECOL 1989;161:1264-6.
5. Nicolaides KH, Peters MT, Vyas S, et al. Relation of rate of urine production to oxygen tension in small-for-gestational age fetuses. AM J OBSTET GYNECOL 1990;162:387-91.
6. Clifford SH. Postmaturity with placental dysfunction. J Pediatr 1954;44:1-19.
7. Manning FA, Baskett TF, Morrison I, et al. Fetal biophysical profile scoring: a prospective study in 1184 high-risk patients. AM J OBSTET GYNECOL 1981;140:289-94.
8. Philipson EH, Sokol EJ, Williams T. Oligohydramnios: clinical associations and predictive value for intrauterine growth retardation. AM J OBSTET GYNECOL 1983;146:271-8.
9. Phelan JP, Platt LD, Yeh SY, et al. The role of ultrasound assessment of amnionic fluid volume in the management of the postdate pregnancy. AM J OBSTET GYNECOL 1985;151:304-8.
10. Gilbert WM, Brace RA. The missing link in amnionic fluid volume regulation: intramembranous absorption. Obstet Gynecol 1989;74:748-53.
11. Brace RA. Fetal blood volume, urine flow, swallowing, and amnionic fluid volume responses to long-term intravascular infusions of saline. AM J OBSTET GYNECOL 1989;161:1049-54.

A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios

Richard Harding, PhD, Stuart B. Hooper, PhD, and Kerry A. Dickson, PhD
Melbourne, Australia

Our aim was to determine the mechanism whereby oligohydramnios causes reduced fetal lung expansion and eventual lung hypoplasia. We studied 20 fetal sheep during 2 to 9 days of oligohydramnios produced by drainage of amniotic and allantoic fluids during the last third of gestation. Oligohydramnios led to a reversible reduction in lung liquid volume of 19.5% within 48 hours. During oligohydramnios tracheal pressure, relative to amniotic pressure, rose by 1.7 mm Hg ($p < 0.001$); pressures also tended to rise in the fetal pleural space and abdomen, relative to amniotic pressure, and to fall in the amniotic sac. Pressure increments, relative to amniotic pressure, which normally occur in the fetal trachea, pleural cavity, and abdomen during nonlabor uterine contractions, were significantly increased by 1.9 to 2.5 mm Hg during oligohydramnios. Oligohydramnios increased flexion of the fetal thoracolumbar spine, quantified as a reduction in the ratio of spinal radius of curvature to spine length (0.76 in controls vs 0.40 after oligohydramnios, $p < 0.001$). In three sets of twins, only the fetus exposed to oligohydramnios was affected. A similar degree of spinal flexion imposed on normal fetal sheep cadavers increased abdominal (1.6 mm Hg), pleural (1.5 mm Hg), and tracheal (2.0 mm Hg) pressures, and caused a significant reduction in fetal lung expansion. We conclude that oligohydramnios in fetal sheep increases spinal flexion, leading to compression of abdominal contents, upward displacement of the diaphragm, and lung compression, favoring loss of fetal lung liquid. These changes, which are accentuated during nonlabor uterine contractions and are reversible, may lead to pulmonary hypoplasia if prolonged. (AM J OBSTET GYNECOL 1990;163:1904-13.)

Key words: Oligohydramnios, fetal lung hypoplasia, fetal posture, uterine contractions, lung liquid

Fetal lung hypoplasia, defined as a reduction in lung weight per unit body weight, is a common consequence of oligohydramnios. In human pregnancy, lung hypoplasia most frequently results from oligohydramnios due to premature rupture of the membranes or to urinary tract abnormalities in the fetus.[1-4] Lung hypoplasia can be produced experimentally by removal of amniotic fluid in rabbits,[5,6] guinea pigs,[8] monkeys,[9] and sheep.[10,11] Although a causal relationship between oligohydramnios and fetal lung hypoplasia has been well established, there remains some controversy as to the mechanism involved.

Fetal lung development is strongly influenced by physical factors,[12] in particular fetal breathing movements and lung expansion by liquid. An impairment of fetal breathing movements[13-15] or a diminished degree of lung expansion[16,17] both result in reduced fetal lung growth. In a recent study in sheep, we showed that prolonged oligohydramnios during the last third of gestation leads to a reduced volume of fetal lung liquid (i.e., reduced lung expansion), whereas fetal breathing movements were not impaired.[11] In an analysis of endocrine and fluid balance responses to oligohydramnios in sheep we have been unable to account for the observed reduction in fetal lung liquid volume.[18]

Although the mechanisms whereby fetal lung liquid volume is reduced during oligohydramnios are not clear, our previous studies indicate that physical factors, such as lung compression, are likely to be involved. Therefore we monitored fetal sheep in utero to determine the effects of short-term, reversible oligohydramnios on pressures that act on the fetal lungs, the volume of lung liquid, and on the flow of this liquid. Oligohydramnios was induced by drainage of amniotic and allantoic fluids, and the reversibility of the fetal effects was assessed by replacement of an appropriate volume of the drained fluids. We also quantified the effects of oligohydramnios on the posture of the fetus in utero. To determine the influence of these changes on the fetal lungs, we examined in fetal cadavers ex utero the effects of imposing changes in posture similar to those induced by oligohydramnios on pressures that act on the lungs and on lung expansion.

From the Department of Physiology, Monash University.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Supported by the National Health and Medical Research Council of Australia and the Sudden Infant Death Research Foundation of Victoria.
Reprint requests: Dr. R. Harding, Department of Physiology, Monash University, Clayton, Australia 3168
6/6/24746

Material and methods

We used 31 fetal sheep from 28 ewes, the mating dates of which were known. Two types of study were performed: (1) measurement of oligohydramnios-induced changes in pressures, lung liquid production, flow and volume, and spinal flexion in chronically prepared fetuses in utero, and (2) measurements of changes in pressures and lung expansion in fetal cadavers during manipulation of fetal posture. The experimental procedures were approved by the Monash University Committee for Ethics in Animal Experimentation.

Chronic preparations. Aseptic surgery was performed with established techniques,[11, 18, 20] on 20 fetuses in 20 pregnant ewes at 101 to 115 days (mean, 107.9 ± 1.0 SEM days) after mating (term, 146 days). In each fetus we implanted ascending and descending catheters into the midcervical trachea; these were subsequently connected externally to form a tracheal "loop" that enabled the measurement of tracheal pressure with a T-connector and fluid flow with a flowmeter.[19, 20] These procedures did not interfere with the function of the fetal upper respiratory tract. Liquid-filled open-tipped or balloon-tipped catheters were implanted into the fetal intrapleural (eight fetuses) and intraperitoneal (eight fetuses) spaces for pressure recording. A catheter was attached to the skin in the midneck region of all fetuses for the measurement of amniotic sac pressure, and another catheter (open or balloon-tipped) was attached to the external surface of the uterus (eight fetuses) to measure maternal intraperitoneal pressure. This was subtracted electronically from amniotic sac pressure to record corrected amniotic sac pressure.

Fine stainless steel electrodes were sewn into the muscle of the fetal diaphragm (six fetuses) and uterus (14 fetuses) to monitor the electromyographic activity of the diaphragm as an index of fetal breathing movements[19, 20] and uterine contractions,[21] respectively. In each preparation, two large diameter silicone rubber catheters (3.2 mm inner diameter, 6.4 mm outer diameter) with protected side ports[11, 18] were attached to the fetal skin at the base of the neck and over the rump; another was implanted within the allantoic sac. These were used to drain amniotic and allantoic fluids by gravity into sterile collection bags placed level with the floor of the sheep's cage.

Study procedure. Fetal surgery was followed by a recovery period of at least 5 days during which no measurements were made. Each study consisted of a control period (1 to 2 days), a period of amniotic and allantoic fluid drainage (2 to 9 days; mean, 3.7 ± 0.3 days), starting at 108 to 138 days' gestation (120 ± 1.2), and a recovery period that followed the reintroduction of drained fluids. The volume replaced was that drained during the first 24 hours of drainage, because this approximates the volume initially contained in the sacs.[11, 18]

In eight experiments we drained fluids a second time, at least five days after restoration of normal amniotic and allantoic fluid volumes. In four of these, the second period of oligohydramnios was used to determine changes in fetal posture that resulted from oligohydramnios. Fetal blood gas values (P_{O_2}, P_{CO_2}, pH), hemoglobin content, and hematocrit were measured daily from samples of carotid arterial blood.

Measurements during in utero studies. Catheters were attached to pressure transducers (Statham P23ID) that were fixed in the same horizontal plane, level with the midpoint of the ewe when standing. Fetal pressures (tracheal, pleural, abdominal) were displayed on a polygraph after subtraction of amniotic sac pressure; 10 mm Hg produced a pen deflection of 20 mm. Amniotic sac pressure (after subtraction of maternal intraperitoneal pressure) was recorded at the same sensitivity. During periods of oligohydramnios, pressures were measured only after the drain catheters had been blocked for 2 to 4 hours, and the amniotic sac catheter flushed with 5 to 10 ml saline solution (to prevent hydrostatic effects). Immediately before each recording period and at 8- to 12-hour intervals, the pressure monitoring circuits were rebalanced and recalibrated. The drift in these circuits was <0.5 mm Hg per 24 hours.

Electromyographic electrodes were connected to differential amplifiers and filters. The fetal diaphragmatic electromyograph was displayed as an integral that reset when full-scale deflection was reached. The number of integrator resets per hour was quantified to give an index of fetal breathing movements. The uterine electromyograph was displayed on the polygraph after filtering to indicate (along with amniotic pressure) nonlabor uterine contractions.

The net efflux of tracheal fluid over 12 to 24 hours was taken as a measure of the production of fetal lung liquid.[19, 20] Because it seemed likely that the flow of lung liquid at the time of nonlabor uterine contractions may be altered by oligohydramnios we also measured net tracheal flow during at least 20 to 35 contractions over 24-hour periods. Lung liquid volume was assessed twice daily in five fetuses by draining liquid from the lower tracheal cannula into a sterile graduated container before returning it to the lungs. During oligohydramnios lung liquid volume was measured only after the amniotic and allantoic drain catheters had been blocked for 2 to 4 hours. In four fetuses, changes in lung liquid volume were measured during two periods of oligohydramnios, and during one period of amniotic and allantoic fluid replacement. In four fetuses, lung liquid volume was also measured by dye dilution[22] during the control periods.

Measurement of spinal curvature. We measured the degree of fetal spinal flexion, between the rump and

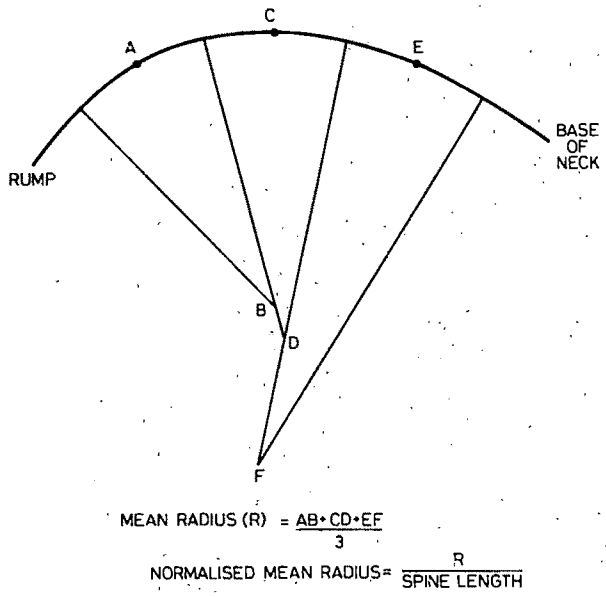


Fig. 1. Technique for quantification of spinal curvature in fetal sheep. Spinal outline between base of neck and rump was divided into four equal segments. Four right bisectors were drawn, and three radii obtained *(AB, CD, EF)*. Mean radius was normalized to permit comparisons between fetuses of different sizes by dividing mean radius by spine length.

upper thorax at postmortem examination while the fetus was in utero. The outline of the spine was traced onto paper and the mean radius of curvature computed; this was normalized by expressing it in relation to the length of the spine over which curvature was measured (Fig. 1). We confirmed the measurements from radiographs of the intact uterus that still contained the fetus after it was removed from the ewe and frozen.

Studies on fetal cadavers ex utero. Fourteen fetuses were studied ex utero <1 hour after death to assess the effects of increased spinal flexion on fetal pressures (n = 6) and lung expansion (n = 12). Six fetuses were implanted with liquid-filled balloon-tipped catheters in the pleural space and abdominal cavity. In each fetus we inserted a catheter into the lower trachea to measure tracheal pressure (n = 6) or to measure the efflux of tracheal fluid (n = 12), taking care to minimize the loss of lung liquid. The fetal upper respiratory tract was not in circuit in the studies on cadavers. However, the absence of the normal resistance presented by the upper respiratory tract[23] would be expected to alter only the time-course of changes in fetal pressures and lung liquid volume caused by spinal flexion. Pressures were recorded on a polygraph while we manipulated the fetuses into three arbitrarily chosen degrees of spinal flexion (mild, moderate, severe). The starting (resting) position was that adopted naturally by the cadaver when it was placed on its side. To measure the change in lung expansion caused by changes in fetal posture we connected the tracheal cannula to a vertical graduated tube. Fluid leaving the lungs entered the tube and its volume was determined at the three stages of spinal flexion. The spinal outline was drawn at each position and the normalized mean radius of curvature was calculated.

Data analysis. In each experiment we measured basal pressures at 5-minute intervals (n = 30 to 35) during the control, oligohydramnios, and recovery periods, excluding episodes of uterine motility. Pressure changes during uterine contractions (n = 20 to 30) were measured at the peak of each contraction. Fetal pressures were measured during apnea or during episodes of fetal breathing movements at end-expiration. Data on pressures and lung liquid production and flow were taken during the last day of the control period, the second day of the oligohydramnios period, and the first 24 hours of the recovery period, starting 6 hours after the return of fluids. We analyzed differences between data obtained during control, oligohydramnios, and recovery periods with the Student t test (paired or unpaired as appropriate) or two-way analysis of variance (ANOVA) followed by a Newman-Keuls multiple range test. Data from cadavers were analyzed with the same tests. Data are presented as mean ± SEM throughout.

Results

Volumes of fluids drained. Volumes of amniotic and allantoic fluids that drained were measured after 24 hours and 48 hours during 15 experiments in 12 sheep. During the first 24 hours of drainage 1149 ± 129 ml had drained from the sacs, of which 1028 ± 111 ml was amniotic fluid. After 48 hours the drained volume was 1634 ± 186 ml, of which 1442 ± 138 ml was amniotic fluid.

Fetal condition throughout experiments. Each fetus was considered to be in good condition throughout the in utero experiments, as indicated by blood gas values, pH, hematocrit, and hemoglobin. Values obtained during the control period (Po_2 24.8 ± 1.1 mm Hg; Pco_2 48.6 ± 2.0 mm Hg; pH 7.335 ± 0.007; Sao_2 61.3 ± 2.8; hemoglobin 9.4 ± 0.3 gm/dl; hematocrit 33.6 ± 2.1%) did not differ significantly from those obtained during the oligohydramnios or recovery periods. Fetal breathing activity was assessed in six experiments (five fetuses); the numbers of diaphragmatic electromyographic integrator resets/hour (arbitrary units) were 21.3 ± 3.0 (control), 26.0 ± 5.7 (oligohydramnios), and 20.5 ± 4.1 (recovery). Although the mean value during oligohydramnios was higher than that during the control period, it was not significantly so ($0.05 < p < 0.10$). However, there was a significant re-

Table I. Basal pressures (mm Hg) in fetal compartments and pressure increases (mm Hg) during nonlabor uterine contractions before, during, and after oligohydramnios in fetal sheep

		Control		*Oligohydramnios*		*Recovery*	
	No.	*Basal*	*Rise*	*Basal*	*Rise*	*Basal*	*Rise*
Amniotic sac	8(8)	−0.15 ± 0.57	—	−1.19 ± 0.45	—	−0.06 ± 0.65	—
pressure	6(4)	—	4.13 ± 0.42	—	4.14 ± 0.70	—	4.58 ± 0.80
Fetal tra-	13(3)	1.27 ± 0.21	—	2.96 ± 0.31	—	1.51 ± 0.16	—
cheal pressure	7(6)	—	1.69 ± 0.49	—	4.01 ± 0.23	—	1.76 ± 0.21
Fetal pleural	8(7)	0.75 ± 0.45	—	0.91 ± 0.47	—	0.60 ± 0.37	—
pressure	9(6)	—	1.17 ± 0.57	—	3.63 ± 0.41	—	1.41 ± 0.32
Fetal ab-	6(4)	1.42 ± 0.38	—	1.82 ± 0.44	—	1.44 ± 0.40	—
dominal pressure	5(4)	—	1.14 ± 0.57	—	2.99 ± 0.71	—	0.69 ± 0.25

Values of n denote numbers of experiments and, in parentheses, numbers of animals.

duction in fetal breathing movements after fluids were replaced ($p < 0.025$).

Basal pressure measurements. Basal pressures in the amniotic sac and in the fetal trachea, pleural cavity, and abdominal cavity during the control, oligohydramnios, and recovery periods are shown in Table I. During oligohydramnios the mean amniotic sac pressure, relative to the ewe's intraperitoneal pressure, was 1.0 ± 0.6 mm Hg lower than that during the control period and 1.1 ± 0.5 mm Hg lower than that during the recovery period, but these changes were not significant ($p < 0.1$, <0.05, respectively). The fetal tracheal pressure, relative to amniotic sac pressure, was significantly elevated by 1.69 ± 0.32 mm Hg during oligohydramnios ($p < 0.001$) from a control value of 1.27 ± 0.21 mm Hg, and was significantly reduced by 1.14 ± 0.19 mm Hg after fluids were returned ($p < 0.001$). Tracheal pressures during the control and recovery periods were not significantly different. Fetal intrapleural and abdominal pressures, relative to amniotic sac pressure, increased slightly during oligohydramnios and declined after the return of fluids, but the pressure changes were not significant (Table I).

Pressures during nonlabor uterine contractions. The mean interval between the start of successive nonlabor uterine contractions increased from 35.6 ± 9.7 minutes in the control period to 46.7 ± 12.6 minutes during oligohydramnios ($p < 0.025$, nine experiments, five animals). During the recovery period the interval between contractions (41.3 ± 4.9 minutes) was not significantly different from that during the oligohydramnios or control periods. The duration of the contractions (nine experiments, six animals) increased from a mean control value of 8.0 ± 0.6 minutes to 9.0 ± 0.8 minutes during oligohydramnios ($p < 0.025$), and decreased significantly to 8.1 ± 0.8 minutes after the return of fluids ($p < 0.01$).

Pressure increments above baseline were measured in the amniotic sac and fetal compartments at the peak of nonlabor uterine contractions during the control, oligohydramnios, and recovery periods (Table I, Fig. 2). Amniotic sac pressure increased during contractions by 4.1 ± 0.5 mm Hg in the control period and was not altered significantly during the oligohydramnios or recovery periods. During uterine contractions fetal tracheal pressure, relative to amniotic sac pressure, increased by 1.7 ± 0.5 mm Hg during the control period, 4.0 ± 0.2 mm Hg during the oligohydramnios period, and 1.8 ± 0.2 mm Hg during the recovery period. That is, oligohydramnios led to an additional increase in tracheal pressure during contractions of 2.3 ± 0.4 mm Hg over the increments that occurred during the control period ($p < 0.001$). This change was reversed when drained fluids were replaced ($p < 0.001$).

The increments in intrapleural pressure, relative to amniotic pressure, during contractions were as follows: control, 1.2 ± 0.5 mm Hg; oligohydramnios, 3.6 ± 0.4 mm Hg; recovery, 1.4 ± 0.3 mm Hg. That is, the pressure increments increased significantly by 2.5 ± 0.4 mm Hg ($p < 0.001$) as a result of oligohydramnios, and decreased after drained fluids were returned ($p < 0.001$). Fetal abdominal pressure also increased above amniotic pressure levels during nonlabor uterine contractions during the control (1.2 ± 0.4 mm Hg), oligohydramnios (3.0 ± 0.7 mm Hg), and recovery (0.7 ± 0.3 mm Hg) periods. During oligohydramnios, the pressure increments increased significantly by 1.9 ± 0.5 mm Hg ($p < 0.025$); when fluids were returned the pressure increments declined significantly ($p < 0.025$) to control values.

Lung liquid production, flow, and volume. The effects of oligohydramnios on fetal lung liquid production and flow during uterine contractions are shown in Table II). Lung liquid production, measured from net tracheal fluid efflux over at least 12 hours, was not significantly altered by oligohydramnios. Tracheal fluid flow rate was measured during uterine contractions in the same experiments during the second day of fluid drainage. Oligohydramnios led to an increase in the mean rate of fluid efflux (milliliters per hour) of 6.8 ±

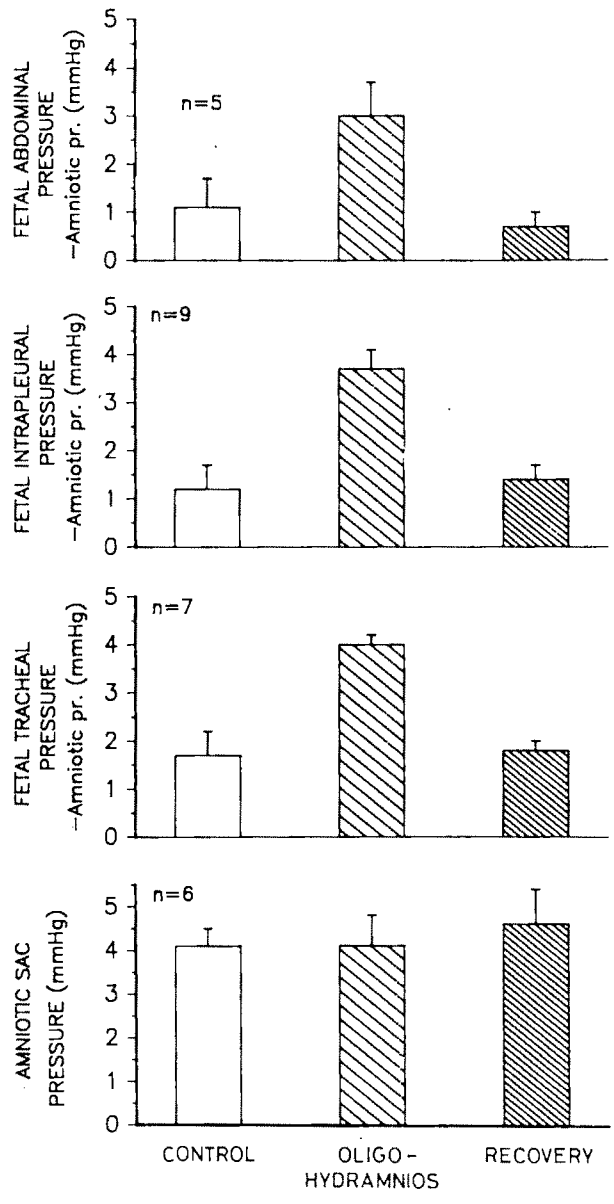


Fig. 2. Increments in fetal pressures and amniotic sac pressure during nonlabor uterine contractions in fetal sheep under control conditions, during oligohydramnios and after replacement of drained fluids (recovery). During oligohydramnios pressure increments in fetal abdomen, pleural space, and trachea rose significantly in relation to amniotic sac pressure. Increments in amniotic sac pressure during contractions did not alter as result of oligohydramnios.

3.1 ml/hour during nonlabor contractions ($p < 0.005$, paired Student t-test) compared with the control period. The rate of efflux during contractions was lower in the recovery period than during oligohydramnios, but the difference was not significant.

The volume of liquid that could be drained from the fetal lungs was measured twice daily during the control, oligohydramnios, and recovery periods (Table II, Fig. 3). Because of normal variability in lung liquid volume

Table II. The effect of oligohydramnios on lung liquid production, tracheal flow during nonlabor uterine contractions, and drained lung liquid volume

	Control	*Oligohydramnios*	*Recovery*
Production (ml/hour, $n = 6$ experiments)	2.82 ± 0.71	3.64 ± 0.58	3.44 ± 0.69
Flow during contractions (ml/hour, $n = 6$ experiments)	5.21 ± 1.03	12.02 ± 3.86	7.67 ± 0.96
Volume (ml, $n = 9$ experiments)	35.3 ± 4.2	28.4 ± 3.9	—
Volume (ml, $n = 5$ experiments)	—	31.2 ± 4.6	43.9 ± 6.9

Data on lung liquid production and flow were obtained in four fetuses; data on lung liquid volume were obtained in a separate group of five fetuses.

with time, a mean value was obtained for each fetus from the four values obtained over 2 days that preceded oligohydramnios, during the first 2 days of oligohydramnios, and during the 2 days after the replacement of fluids. During nine periods of oligohydramnios in five fetuses, starting at 112 to 128 (119 ± 2) days' gestation, there was a reduction of drained lung liquid volume from 35.3 ± 4.2 ml to 28.4 ± 3.9 ml ($p < 0.025$). Replacement of amniotic and allantoic fluids at 121 ± 3 days' gestation led to an increase in lung liquid volume from 31.2 ± 4.6 ml to 43.9 ± 6.9 ml (five experiments, five fetuses; $p < 0.001$). The volume of lung liquid drained was 84.2% ± 8.6% of the volume obtained by the dye-dilution method (nine experiments, four fetuses).

Spinal curvature in utero. Spinal curvature was measured in seven fetuses (aged 123 to 130 days) that had not been subjected to oligohydramnios, and in seven fetuses (aged 125 to 138 days) that had been subjected to 2 to 9 days of oligohydramnios (Table III, Fig. 4). When the mean radius of curvature was normalized for spine length, values obtained in fetuses killed during oligohydramnios (0.40 ± 0.02) were significantly smaller than those in control fetuses (0.76 ± 0.07, $p < 0.001$). In each of three sets of twins included in the group described above, the spinal radius was less in the fetus exposed to oligohydramnios than that in the control twin (Table III).

Studies on fetal cadavers. The aim of these studies was to measure the effects of imposed changes in spinal flexion on pressures that affect the fetal lungs and on lung expansion. In the resting position, pressures in the abdomen, pleural cavity, and trachea were equal to

Table III. The effect of oligohydramnios on spinal radius of curvature normalized for spine length in fetal sheep in utero

	Control	*Oligohydramnios*
No. of fetuses	7	7
Fetal age (days)	130.7 ± 1.6	126.8 ± 1.0
Spinal radius (R/L)	0.76 ± 0.07	0.40 ± 0.02
Days of oligohydramnios	—	5.7 ± 1.0
No. of twin fetuses	3	3
Fetal age (days)	126, 129, 130	126, 129, 130
Spinal radius (R/L)	0.87, 0.55, 0.89	0.34, 0.42, 0.43
Days of oligohydramnios	—	5, 7, 9

R, Radius of curvature; *L*, spine length. Data on three sets of twins included in the group of seven fetuses *(upper)* are set out separately *(lower)*.

atmospheric pressure. Pressure changes in six fetuses (aged 123 to 141 days) at the three stages of spinal flexion are shown in Table IV. The mean pressures in each compartment increased incrementally when the spine was increasingly flexed; however, the differences between pressures during mild and moderate degrees of flexion were not significantly different. At each level of spinal flexion, the mean pressures measured in the trachea tended to be greater than those in the other fetal compartments, although the differences were not significant.

In a separate study, changes in fetal liquid lung volume were studied in 12 fetal cadavers between 123 and 141 days' gestation (Fig. 5, Table IV). At the resting position a pressure head of 1 to 2 cm water was created in the tracheal liquid. With increasing degrees of spinal flexion (mild, moderate, severe) increasing amounts of luminal liquid left the lungs via the trachea in each fetus. With mild, moderate, and severe flexion, the volume leaving the lungs represented 5.8%, 11.0%, and 18.0%, respectively, of the initial volume of lung liquid (determined by drainage); these volumes were significantly different from each other. There was no significant effect of gestational age on the change in lung liquid volume during spinal flexion (Fig. 5).

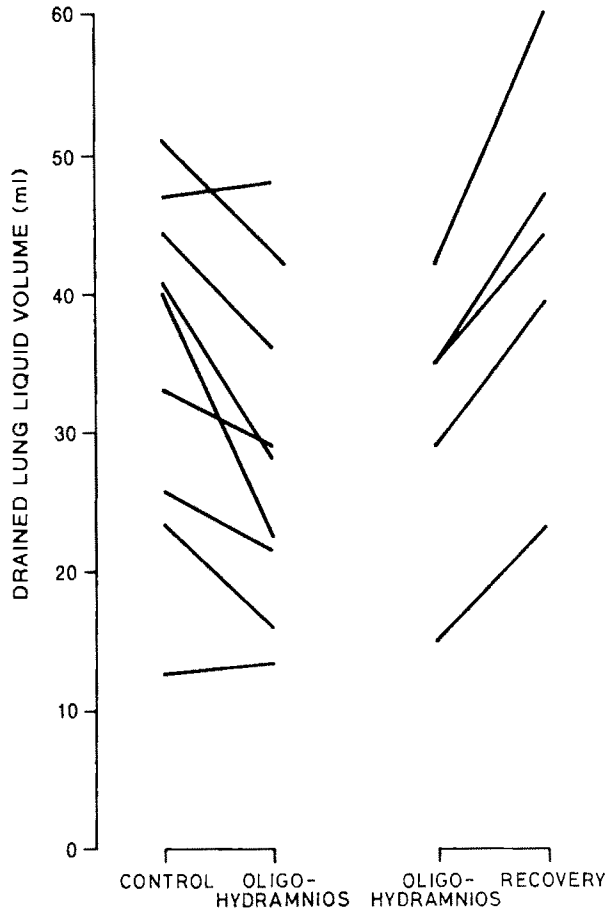


Fig. 3. Changes in drained lung liquid volume in five fetal sheep in response to oligohydramnios and to replacement of drained amniotic and allantoic fluids (i.e., recovery). In four of five fetuses there were two periods of oligohydramnios. Each data point is mean of four values obtained over 48 hours.

Comment

In our previous study[11] we showed that prolonged oligohydramnios resulted in reduced lung expansion in fetal sheep, an observation that we have now confirmed during short-term oligohydramnios. A reduction in fetal lung expansion, if prolonged, is known to result in lung hypoplasia,[16, 17] and is the most likely explanation for oligohydramnios-induced lung hypoplasia. We now present data that show a mechanism whereby oligohydramnios leads to reduced fetal lung expansion and that the effects are established quickly and can be readily reversed by replacement of fluids into the fetal sacs. Our observations indicate that oligohydramnios causes a rise in fetal pulmonary "airway" pressure, relative to amniotic sac pressure, principally as a result of an alteration in fetal posture (i.e., increased spinal flexion) imposed by a reduction in uterine volume. Our data also indicate that in twin pregnancies only the fetus without an adequate volume of surrounding fluid is affected.

The increase in tracheal fluid pressure, relative to amniotic pressure, that occurs during oligohydramnios would be expected to lead to an increased efflux of liquid from the lungs via the trachea. Such a flow of liquid would be very difficult to detect under basal conditions because of the normally large variability of tracheal flow in the fetus.[20] However, we were able to detect an increased rate of efflux of fluid during nonlabor uterine contractions when the pressure difference between the lungs and amniotic sac increases for several minutes. The measurement of an increased tracheal pressure also indicates that the fetus is able to resist, at least partially, the loss of luminal liquid from

Table IV. *Upper,* increments in fetal pressures during increasing degrees of spinal flexion in six fetal sheep cadavers ex utero (mean age, 134 ± 3.2 days); *lower,* efflux of lung liquid via the trachea with increasing degrees of spinal flexion in 12 fetal sheep cadavers (mean age, 132.2 ± 2.1 days)

	Resting	*Mild flexion*	*Moderate flexion*	*Severe flexion*
Tracheal pressure (mm Hg)	—	1.54 ± 0.16	1.98 ± 0.35	3.15 ± 0.47
Pleural pressure (mm Hg)	—	1.18 ± 0.25	1.52 ± 0.31	2.50 ± 0.45
Abdominal pressure (mm Hg)	—	1.30 ± 0.09	1.58 ± 0.14	2.70 ± 0.49
R/L	0.65 ± 0.09	0.45 ± 0.04	0.38 ± 0.02	0.34 ± 0.02
Reduction in lung liquid volume (ml)	—	2.5 ± 0.4	5.0 ± 0.6	8.2 ± 0.8
R/L	0.77 ± 0.07	0.49 ± 0.03	0.41 ± 0.01	0.35 ± 0.01

Normalized spinal radius (R/L) is given for each fetal position.

its lungs. The loss of lung liquid would be opposed (but could not be prevented) by the upper airway, which normally offers a resistance to the efflux of tracheal liquid, particularly in the absence of fetal breathing movements.[23] It is possible that an increase in the resistance of the upper airway during uterine contractions may have contributed to the observed increase in tracheal pressure.

Our data on changes in fetal posture that result from oligohydramnios and from fetal cadavers subjected to similar postural changes allow us to propose an explanation for increased tracheal pressure and reduced lung liquid volume associated with oligohydramnios. In fetal cadavers with liquid-filled lungs, increased flexion of the spine between the upper thorax and the rump, caused graded increases in pressure in the abdomen, pleural cavity, and trachea. Most of this flexion occurs in the abdominal segments. We propose that as the abdominal contents become compressed the diaphragm is displaced into the thorax, thereby compressing the lungs. In cadavers we observed that this effect was increased by apposition of the upper hind limbs to the abdominal wall; this postural feature was noted in some of the fetuses post mortem after oligohydramnios.

In fetuses killed during oligohydramnios the degree of spinal flexion, quantified as the ratio of mean spinal radius to spine length, was approximately equal to moderate spinal flexion that we imposed on cadavers. This produced a rise in tracheal pressure of 2.0 mm Hg, which was slightly greater than the pressure rise measured in the fetal trachea in utero, in the absence of uterine muscle activity. The pressure increments in the abdomen and pleural space of cadavers during moderate flexion were also greater than the nonsignificant increments in these compartments in vivo during oligohydramnios. These differences between in utero and ex utero measurements are probably a result of those in cadavers being made in a closed system (i.e., lung liquid could not escape via the trachea), whereas in utero the fetal trachea can communicate with the amniotic sac. Moderate spinal flexion in cadavers caused a loss of 5 ml of luminal liquid from the lungs. This was less than we measured during 48 hours of oligohydramnios, possibly because the lungs of cadavers are less expanded, due to unavoidable escape of lung liquid after death of the fetuses. The volumes of fluid that we subsequently drained from the lungs of cadavers supports this. However, the studies on fetal cadavers clearly show that posture is an important determinant of basal lung capacity, as it is in the adult.[24] In particular, our studies show that increasing spinal flexion, similar to that induced by oligohydramnios, can lead to compression of the fetal lungs. In the absence of an upper airway it is to be expected that this would lead to reduced lung expansion, but even with an intact, functioning upper airway and continuing fetal breathing movements, a reduction in fetal lung expansion must occur, although the time course may be longer.

Our measurement of a nonsignificant reduction in amniotic sac pressure is in agreement with our earlier study.[11] In human pregnancy, a reduction in amniotic sac pressure has been reported during oligohydramnios,[25] which may be expected to accentuate the driving force favoring loss of liquid from the lungs. However, a fall in amniotic sac pressure in the absence of fetal postural changes would be expected to be accompanied by a similar reduction in fetal pressures. That fetal pressures did not fall but tended to rise indicates that some independent rise in pressures occurred within the fetus. We propose that this is a result of an imposed change in fetal posture.

We have shown that the effects of oligohydramnios on the fetal lungs are increased by nonlabor contractions of the uterus, which are a normal feature of the latter half of pregnancy in many species including human beings.[21, 26, 27] Although they are of low intensity (typically <5 mm Hg) they are known to exert effects on the fetus, including alteration of thoracic dimensions,[28] reduced fetal oxygenation,[29] and reductions in uterine blood flow.[28] Ultrasonographic observations on fetal pygmy goats showed that these contractions also cause increasing degrees of spinal flexion.[27] This accords with our observations of increased pressures in the fetal abdomen, pleural cavity, and trachea, particularly during oligohydramnios. Even when normal volumes of amniotic and allantoic fluids are present, uter-

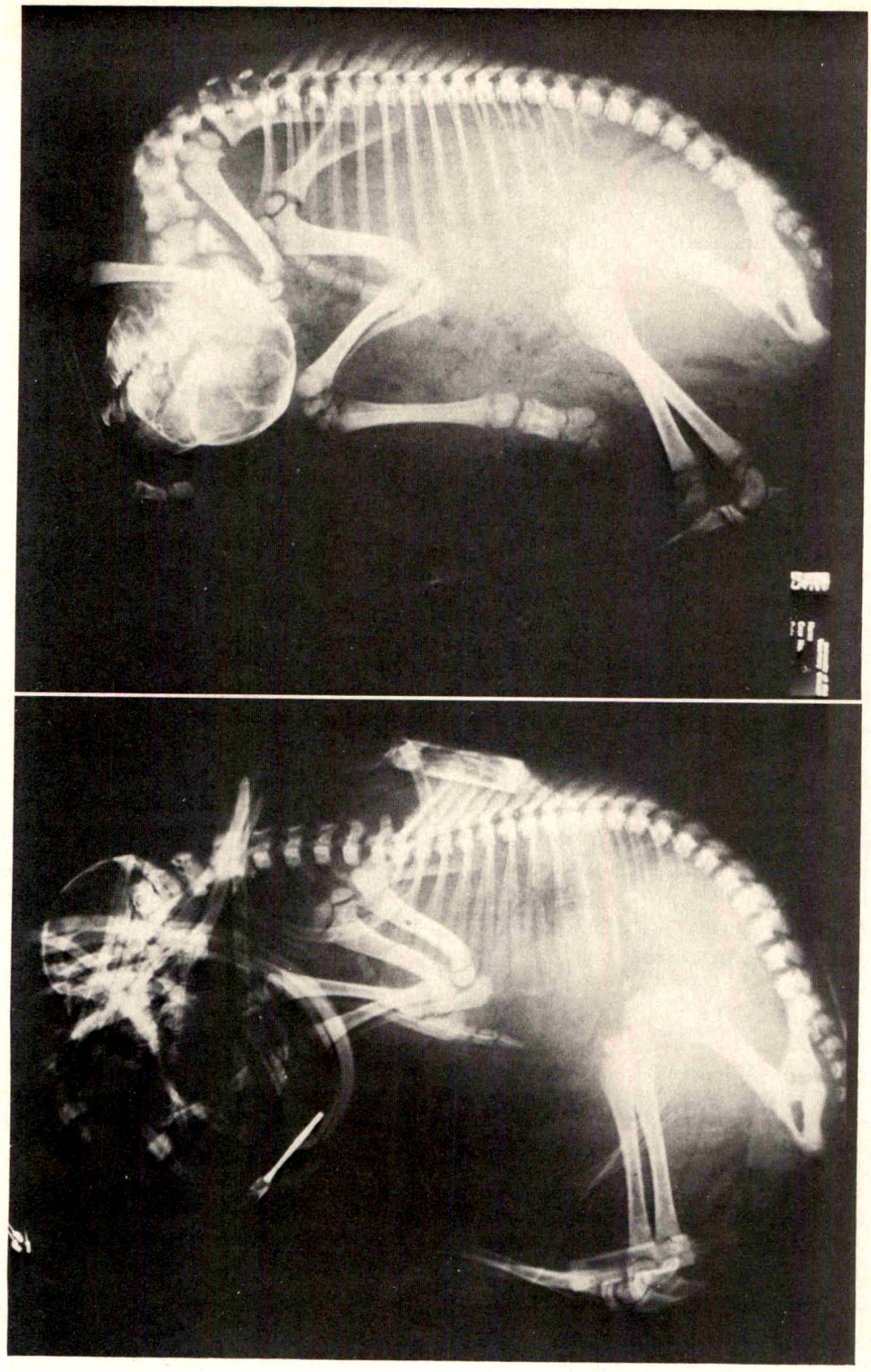


Fig. 4. Radiographs of fetal sheep in utero after removal from ewe. *Upper*, control fetus aged 135 days. *Lower*, fetus aged 129 days after 9 days of oligohydramnios. Note greater degree of spinal flexion in fetus in presence of oligohydramnios.

ine contractions are associated with increased pulmonary airway pressure and increased flow of liquid from the lungs, particularly when the contractions coincide with episodes of fetal breathing movements.[30] The efflux of lung liquid during contractions is increased during oligohydramnios presumably because of the greater pressure difference between the lungs and amniotic sac; the contractions are also slightly longer. Whether

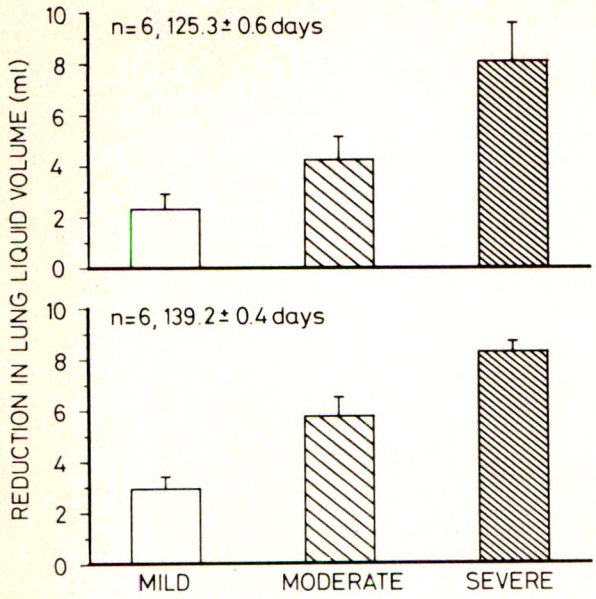


Fig. 5. Reduction in lung liquid volume in 12 fetal sheep cadavers ex utero at two age groups (*upper*, 123 to 127 days and *lower*, 136 to 141 days) in response to three stages of imposed spinal flexion (mild, moderate, severe). In both groups, increasing spinal flexion led to increased loss of liquid from lungs via trachea.

this additional loss of lung liquid contributes to the reduction in lung liquid volume during oligohydramnios cannot be determined at present. That the fetus is able to resist the compressive effects of these contractions, at least partially, is shown by the measured increases in tracheal pressure. This resistance is likely to be a result of fetal muscular activity in the upper respiratory tract, because we observed in one fetus a greatly increased efflux of lung liquid via the trachea during contractions after it was inadvertently killed in utero during oligohydramnios.

The effects of oligohydramnios on fetal pressures were evident within 12 to 24 hours of the start of drainage of amniotic and allantoic fluids, and a reduction of lung liquid volume was measured over the first 48 hours. This is to be expected if the observed effects are indeed a result of the physical consequences of reduced uterine dimensions. Similarly, it is to be expected that the fetal pressure and lung volume changes should be reversible, which we found them to be. Normal fetal pressures (basal and during uterine contractions) were recorded within 2 to 4 hours of reexpanding the fetal fluid sacs. It is of interest that an increase in the volume of amniotic fluid above normal in pregnant sheep will reduce the extent of changes in thoracic dimensions during nonlabor uterine contractions.[31]

Lung liquid production was not affected by short-term oligohydramnios during the present study and therefore cannot have contributed to the observed changes in pulmonary pressures or volume. This is in agreement with our earlier study[11] in which there was no reduction in lung liquid production until after 48 hours of oligohydramnios. It is likely that the reduced lung liquid production we previously observed during prolonged oligohydramnios was principally a result of reduced lung growth and was not a response to alterations in the endocrine or fluid-balance status of the animals.[18]

We conclude that oligohydramnios in ovine pregnancy can lead to an increased pressure gradient between the pulmonary airways of the fetus and the amniotic sac, favoring the loss of lung liquid via the trachea, and a reduction in lung expansion. These changes are probably maintained by increased spinal flexion imposed on the fetus by a reduced uterine volume, and are augmented during recurring nonlabor contractions of the uterus. The effects on the fetus are reversible and support the clinical management of the fetus exposed to oligohydramnios by intraamniotic infusion of fluids.[32]

Expert technical assistance of Ms. K. Billings, Ms. J. Norman, and Mr. A. Satragno is gratefully acknowledged. Radiography was kindly performed by Dr. R. Lavelle, Veterinary Clinical Centre, University of Melbourne.

REFERENCES

1. Thomas IT, Smith DW. Oligohydramnios, cause of the nonrenal features of Potter's syndrome, including pulmonary hypoplasia. J Pediatr 1974;84:811-4.
2. Wigglesworth JS, Desai R, Guerrini P. Fetal lung hypoplasia: biochemical and structural variations and their possible significance. Arch Dis Child 1981;56:606-15.
3. Nimrod C, Verela-Gittings F, Machin G, Campbell D, Wesenburg R. The effect of very prolonged membrane rupture on fetal development. Am J Obstet Gynecol 1984; 148:540-3.
4. Thibeault DW, Beatty EC, Hall RT, Bowen SK, O'Neill DH. Neonatal pulmonary hypoplasia with premature rupture of fetal membranes and oligohydramnios. J Pediatr 1985;107:273-7.
5. Adzick NS, Harrison MR, Glick PL, Villa RL, Finkbeiner W. Experimental pulmonary hypoplasia and oligohydramnios: relative contributions of lung fluid and fetal breathing movements. J Pediatr Surg 1984;19:658-63.
6. King JC, Mitzner W, Butterfield AB, Queenan JT. Effect of induced oligohydramnios on fetal lung development. Am J Obstet Gynecol 1986;154:823-30.
7. Moessinger AC, Bassi GA, Ballantyne G, Collins MH, James LS, Blanc WA. Experimental production of pulmonary hypoplasia following amniocentesis and oligohydramnios. Early Hum Dev 1983;8:343-50.
8. Moessinger AC, Collins MH, Blanc WA, Rey HR, James LS. Oligohydramnios-induced lung hypoplasia: the influence of timing and duration in gestation. Pediatr Res 1986;20:951-4.
9. Hislop A, Fairweather DVI, Blackwell RJ, Howard S. The

effect of amniocentesis and drainage of amniotic fluid on lung development in Macaca fascicularis. Br J Obstet Gynaecol 1984;91:835-42.
10. Moessinger AC, Fewell JE, Stark RI, et al. Lung hypoplasia and breathing movements following oligohydramnios in fetal lambs. In: Jones CT, Nathanielsz PW, eds. The physiological development of the fetus and newborn. London: Academic Press, 1985:293-8.
11. Dickson KA, Harding R. Decline in lung liquid volume and secretion rate during oligohydramnios in fetal sheep. J Appl Physiol 1989;67:2400-7.
12. Kitterman JA. Physical factors and fetal lung growth. In: Johnston BM, Gluckman PD, eds. Respiratory control and lung development in the fetus and newborn. Ithaca, New York: Perinatology Press, 1986:63-85.
13. Wigglesworth JA, Desai R. Effect on lung growth of cervical cord section in the rabbit fetus. Early Hum Dev 1979; 3:51-65.
14. Liggins GC, Vilos GA, Campos GA, Kitterman JA, Lee CH. The effect of spinal cord transection on lung development in fetal sheep. J Develop Physiol 1981;3:267-74.
15. Liggins GC, Vilos GA, Campos GA, Kitterman JA, Lee CH. The effect of bilateral thoracoplasty on lung development in fetal sheep. J Develop Physiol 1981;3:275-82.
16. Alcorn D, Adamson TM, Lambert TF, Maloney JE, Ritchie BC, Robinson PM. Morphological effects of chronic tracheal ligation and drainage in the fetal lamb. J Anat 1977;123:649-60.
17. Moessinger AC, Harding R, Adamson TM, Singh M, Kiu G. Local vs systemic factors at play in fetal lung growth and maturation [Abstract]. Pediatr Res 1989;25:56.
18. Dickson KA, Hooper SB, McMillen IC, Harding R. Endocrine and fluid balance responses to amniotic and allantoic fluid loss in sheep. Am J Physiol 1990;259: R745-52.
19. Dickson KA, Harding R. Restoration of lung liquid volume following its acute alteration in fetal sheep. J Physiol 1987;385:531-43.
20. Harding R, Sigger JN, Wickham PJD, Bocking AD. The regulation of flow of pulmonary fluid in fetal sheep. Respir Physiol 1984;57:47-59.
21. Harding R, Poore ER, Bailey A, Thorburn GD, Jansen CAH, Nathanielsz PW. Electromyographic activity of the nonpregnant and pregnant sheep uterus. Am J Obstet Gynecol 1982;142:448-57.
22. Hooper SB, Dickson KA, Harding R. Lung liquid secretion, flow and volume in response to moderate asphyxia in fetal sheep. J Develop Physiol 1988;10:473-85.
23. Harding R, Bocking, Sigger JN. Upper airway resistances in fetal sheep: the influence of breathing activity. J Appl Physiol 1986;40:160-6.
24. Agostini E, Hyatt RE. Static behavior of the respiratory system. In: Fishman AP, Macklem PT, Mead J, Geiger SR, eds. Handbook of Physiology, section 3, vol III. Bethesda: American Physiological Society, 1986:113-30.
25. Nicolini U, Fisk NM, Rodeck CH, Talbert DG, Wigglesworth JS. Low amniotic pressure in oligohydramnios: is this the cause of pulmonary hypoplasia. Am J Obstet Gynecol 1989;161:1098-101.
26. Mulder EJH, Visser GHA. Braxton Hicks' contractions and motor behavior in the near-term human fetus. Am J Obstet Gynecol 1987;156:543-9.
27. Scheerboom JEM, Taverne MAM. A study of the pregnant uterus of the ewe and the goat using real-time ultrasound scanning and electromyography. Vet Res Commun 1985;9:45-56.
28. Harding R, Poore ER. The effects of myometrial activity on fetal thoracic dimensions and uterine blood flow during late gestation in the sheep. Biol Neonate 1984;45:244-51.
29. Jansen CAM, Krane EJ, Thomas AL, Beck NFG, Lowe KC, Joyce P, Parr M, Nathanielsz. Continuous variability of fetal PO_2 in the chronically catheterized fetal sheep. Am J Obstet Gynecol 1979;134:776-83.
30. Harding R, Bocking AD, Sigger JN. Influence of upper respiratory tract on liquid flow to and from fetal lungs. J Appl Physiol 1986;61:68-74.
31. Nathanielsz PW, Figueroa JP, El Badry A, et al. Myometrial activity—control and effects. In: Jones CT, Nathanielsz PW, eds. The physiological development of the fetus and newborn. London: Academic Press, 1985:355-62.
32. Imanaka M, Ogita S, Sugawa T. Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes. Am J Obstet Gynecol 1989;161:102-6.

Soluble factors produced by isolated first-trimester chorionic villi directly inhibit proliferation of T cells

Richard K. Silver, MD, Jane M. Turbov, BA, Judith A. Beaird, BS, and Joseph Golbus, MD

Evanston, Illinois

Supernatants from human trophoblast cultures have been reported to be both suppressive and stimulatory of mitogen-induced T-lymphocyte transformation. These discordant observations have been attributed to methodologic differences among laboratories, but may instead relate to inadvertant cellular contamination during long-term culture. Retrieval of chorionic villi for genetic analysis has provided an opportunity to evaluate the immunoregulatory activity of the isolated villus preparation, unencumbered by extended culture techniques. Primary cytotrophoblast cultures were established from karyotypically and clinically normal pregnancies and the supernatants were recovered. Allogenic mononuclear cells, stimulated by phytohemagglutinin, were uniformly inhibited by trophoblast supernatant (mean inhibition, 34.3%). This inhibition was verified in one-way, mixed lymphocyte cultures and suppression of T cells occurred in a dose-dependent fashion over a range of trophoblast supernatant concentrations. No evidence of suppressor T-cell induction by trophoblast supernatant was noted with the use of both phenotypic studies of lymphocytes incubated with trophoblast supernatant and a functional suppressor T-cell inducer assay. We believe that the immunoregulatory properties of trophoblast supernatants identified with the use of these methods more closely approximate the paracrine function of the in situ villus. (Am J Obstet Gynecol 1990;163:1914-9.)

Key words: Trophoblast, lymphocytes, immunosuppression

There exists considerable evidence that maternal immunologic tolerance of the fetal allograft is, in part, dependent on the intervening trophoblast barrier. The influence of this fetally derived tissue is likely diverse and includes both passive and active immunoregulation.[1-4] The presumed "nonantigenicity" of the outer syncytiotrophoblast and its ability to bind and neutralize offending antibodies are two examples of passive immunoregulation.[5,6] In contrast, synthesis and release of immunologically potent mediators may modulate both the systemic host response and the local intrauterine environment.[7,8] Contemporary investigations that have focused on mediator release by cultured trophoblast cells have yielded conflicting results. For example, Yagel et al.[9] described a predominantly cytotrophic influence on lymphocyte proliferation by both trophoblast cells and their supernatants, whereas Sanyal et al.[10] observed immunosuppression with similar methods. Discordant observations have also been noted in studies with murine placental extracts presumed to contain distinct molecules that promote suppression or stimulation, depending on their relative concentrations.[11]

Whereas it is possible that the in situ human trophoblast could be both suppressive and stimulatory of maternal immune responses, these contradictory observations might instead be related to differences in the specific methods of prior experiments.[9,10] Retrieval of trophoblast material from pregnancies undergoing elective termination might inadvertantly include samples from gestations destined to develop clinical abnormalities (e.g., intrauterine growth retardation), making inferences regarding normal trophoblast immune function inappropriate. The possibility that some aneuploid pregnancies might be included also exists. In addition, reliance on relatively long-term culture techniques could lead to nontrophoblast cell contamination or even dedifferentiation of trophoblast cells cultured by repeated passage. In the immunologic studies by Yagel et al., trophoblast cell lines were maintained for up to 100 days (by serial in vitro passages at 4- to 8-day intervals).

To avoid these potentially confounding issues and to ensure that the villus samples retrieved for study were associated with genetically and clinically normal pregnancies, we used short-term cultures of karyotypically normal, isolated chorionic villi obtained for prenatal diagnosis from otherwise low-risk patients. Our specific objective was to use this trophoblast source to evaluate

From the Divisions of Maternal-Fetal Medicine and Rheumatology, Departments of Obstetrics and Gynecology and Medicine, Northwestern University Medical School, Evanston Hospital.
Supported in part by a Dee and Moody Grant, Evanston Hospital.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Richard K. Silver, MD, Evanston Hospital, Room 1600, 2650 Ridge Ave., Evanston, IL 60201.
6/6/24770

Table I. Selected characteristics of the chorionic villus sampling procedures from which trophoblast supernatant samples were obtained

Immunologic assays performed	*Number of trophoblast supernatant samples used*	*Route of chorionic villus sampling (Cervical/Abdominal)*	*Gestational age (wk) (mean ± SD)*
PHA-mitogenesis (Fig. 1)	34	15/19	10.0 ± 1.0
PHA-mitogenesis, dose-response (Fig. 2)	3	1/2	9.4 ± 0.3
Mixed lymphocyte culture (Fig. 3)	10	7/3	9.8 ± 0.9
Suppressor T-cell inducer assay (Fig. 4)	8	4/4	10.7 ± 1.1

PHA, Phytohemagglutinin.

the influence of trophoblast supernatants on selected assays of cellular immune function.

Material and methods

Retrieval of trophoblast supernatants. Chorionic villus tissue was recovered for cytogenetic analysis (after informed consent with a protocol approved by the Institutional Review Board) by either the transabdominal or transcervical route between 8 to 12 weeks' gestation, with sampling methods previously reported.[12] Preparation and culturing of the specimens was accomplished with methods adapted from Simoni et al.[13] and Niazi et al.,[14] designed to minimize the possibility of maternal or nontrophoblast cell contamination.[15] Briefly, recovered villi were meticulously cleaned under an inverted microscope, then reduced to their mesenchymal core by enzyme digestion (trypsin followed by collagenase). This latter step removes the outer syncytiotrophoblast layer along with any adherent nontrophoblast cells (e.g., decidual cells, red blood cells, and macrophages) that remain after the initial dissection. The samples were then centrifuged and resuspended in Chang's growth medium (Hana Biologics, Irvine, Calif.). Primary cultures were refed with fresh media at 24 hours, after which the media were not changed. Cultures were harvested on day 6, at which time there were approximately 2×10^3 cells per culture well. The supernatants were recovered and frozen at −20° C so that the clinical outcome of each pregnancy would be known before inclusion of the sample in the immunologic assays. The supernatants from 55 chorionic villus specimens (obtained between November 1988 and October 1989) from completed, karyotypically and clinically normal pregnancies were selected randomly to be used in the experiments described here. Selected characteristics of the chorionic villus sampling procedures from which these trophoblast supernatant samples were retrieved are summarized in Table I.

Mitogen-stimulated mononuclear cell assays. To determine the effect of trophoblast supernatant on mitogen-induced T-cell transformation, mononuclear cells, isolated from the blood of healthy, nonpregnant adult volunteers and suspended at 2×10^5 cells/ml were cultured for 3 and 4 days with 0.2 μg/ml of phytohemagglutinin (Sigma Chemical Co., St. Louis). Trophoblast supernatant (25% concentration) or an equal volume of control media (growth media that was innoculated into empty culture wells at the initiation of the villus culture) was added at the onset of mitogen stimulation. After an 18-hour tritiated thymidine exposure, cultures were harvested and liquid scintillation counting was performed. Viability testing (by trypan blue exclusion) was >85% in this and all other experiments. The means and SE of all triplicates were calculated and the degree of suppression was expressed as a ratio of the mean counts per minute of trophoblast supernatant experiments over the mean counts per minute of control experiments. Additional assays were also performed with higher and lower concentrations of trophoblast supernatants (1%, 5%, 10%, and 50%) in an attempt to identify a dose-response relationship. T-cell mitogen experiments were repeated with aliquots of heat-inactivated trophoblast supernatants (30 minutes in a 56° C shaking water bath).

One-way, mixed lymphocyte cultures. To confirm that the immunosuppression observed in the mitogen experiments was T-cell specific, 5-day mixed lymphocyte cultures were performed with mononuclear cells from two human lymphocyte antigen–incompatible donors. Responders and stimulators (treated with mitomycin C) were combined in a 5 : 1 ratio (to maximize the detection of immunosuppression). Trophoblast supernatant or control media was added at a 25% concentration to the culture wells at initial coincubation. Cultures were pulsed with tritiated thymidine and then terminated and counted as previously described. Stimulation indexes were calculated for all triplicates after subtraction of background counts derived from autologous combinations. Results were expressed as a ratio of the trophoblast supernatant and control media stimulation index values to quantitate suppression.

Suppressor T-cell inducer assay. To determine whether the observed suppression involved the induction of suppressor T cells by trophoblast supernatant, a two-stage experiment was performed. Initially, mononuclear cells were incubated with 25% trophoblast su-

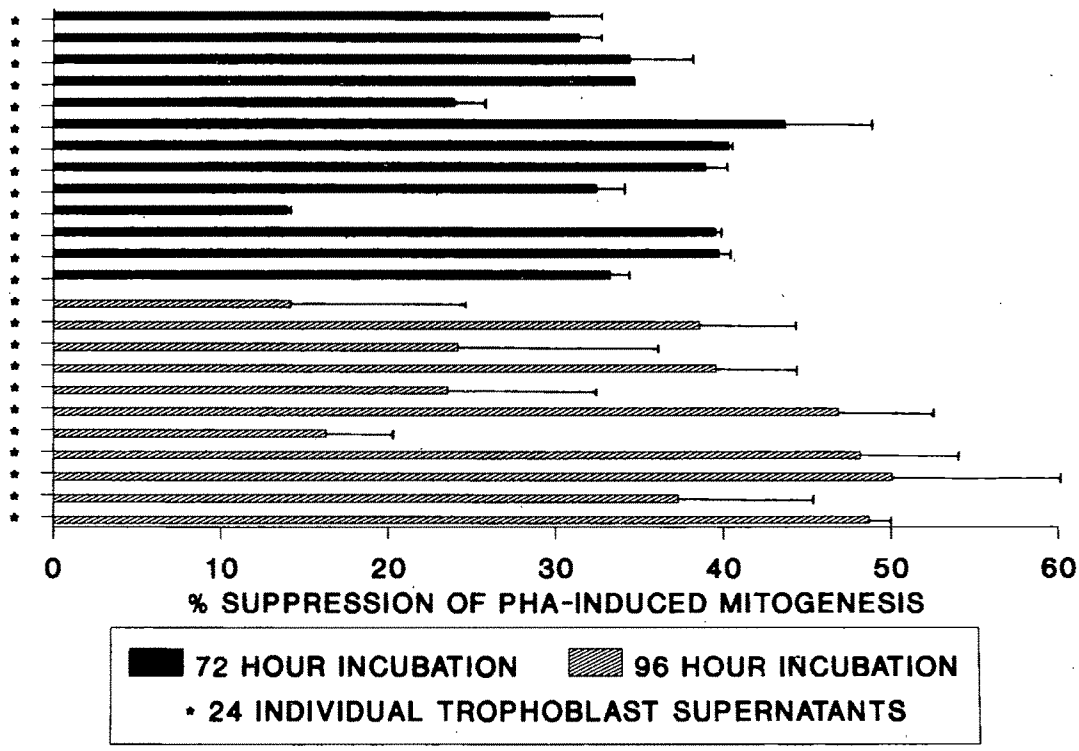


Fig. 1. Suppression of T-cell mitogenesis by trophoblast supernatants. Results from two periods of incubation with 24 different trophoblast supernatant samples. Means and corresponding SEs shown. *PHA*, phytohemagglutinin.

pernatant or media only for 3 days. These two groups of cells were removed by vigorous pipeting, rinsed with media, and then washed and exposed to mitomycin C. In the second stage, both the trophoblast supernatant–treated and control-treated cells (1×10^5 cells/ml) were incubated for 72 additional hours with the same number of fresh, untreated cells and 0.5 μg/ml of phytohemagglutinin. These cultures were pulsed and harvested as previously described. In addition, separate aliquots of the trophoblast supernatant–treated and control-treated cells were analyzed by flow fluorocytometry (Bectin-Dickenson) with antibody labeling to detect any change in the percentage of suppressor $T8^+$ cells (as a consequence of trophoblast supernatant exposure).

Statistical analysis. Group means were analyzed by the two-tailed Student *t* test or analysis of variance. The SEM of each triplicate set is depicted in each figure. A *p* value <0.05 was considered as significant.

Results

Phytohemagglutinin-induced proliferation of T cells was uniformly inhibited in 24 separate trophoblast supernatant experiments with either 3-day or 4-day exposures (Fig. 1). Suppression was observed in all additional supernatants used in these experiments (data not shown). On average, a 34% decrease was observed (range, 14 to 50) and heat inactivation did not alter this finding (mean suppression induced by heat-inactivated trophoblast supernatant = 33%; range, 20 to 44; $n = 10$). Fig. 2 shows a clear dose response, with maximal suppression occurring at the 50% trophoblast supernatant concentration ($p < 0.001$ by Friedman analysis of variance). Performance of one-way mixed lymphocyte cultures in the presence of 25% trophoblast supernatant confirmed immunosuppression in all 10 samples tested (Fig. 3).

An evaluation of suppressor T-cell induction with six individual mononuclear cell donors and eight different trophoblast supernatant samples (known to be suppressive of mitogen-induced proliferation) failed to reveal functional evidence of suppressor T-cell induction (Fig. 4). In fact, moderate enhancement of phytohemagglutinin-induced transformation was noted in most of these experiments. Although an overall increase in the percentage of phenotypic suppressor T cells was observed by fluoresence antibody binding (Table II), this change was also seen in cells exposed to media alone, suggesting that it was a temporal artifact of mononuclear cell culture.

Comment

With the trophoblast source and culture techniques described in this study, trophoblast supernatant con-

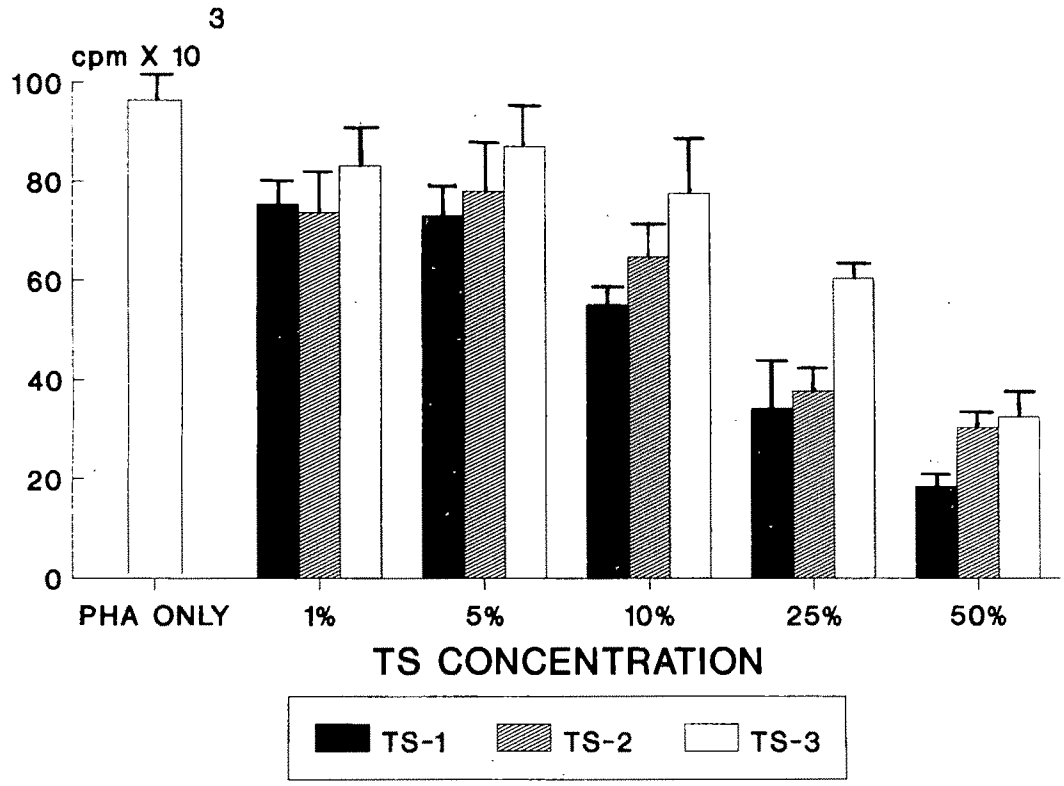


Fig. 2. Dose-response of suppression of T-cell mitogenesis with three different trophoblast supernatant *(TS)* samples. Means and corresponding SEs shown. *PHA*, phytohemagglutinin.

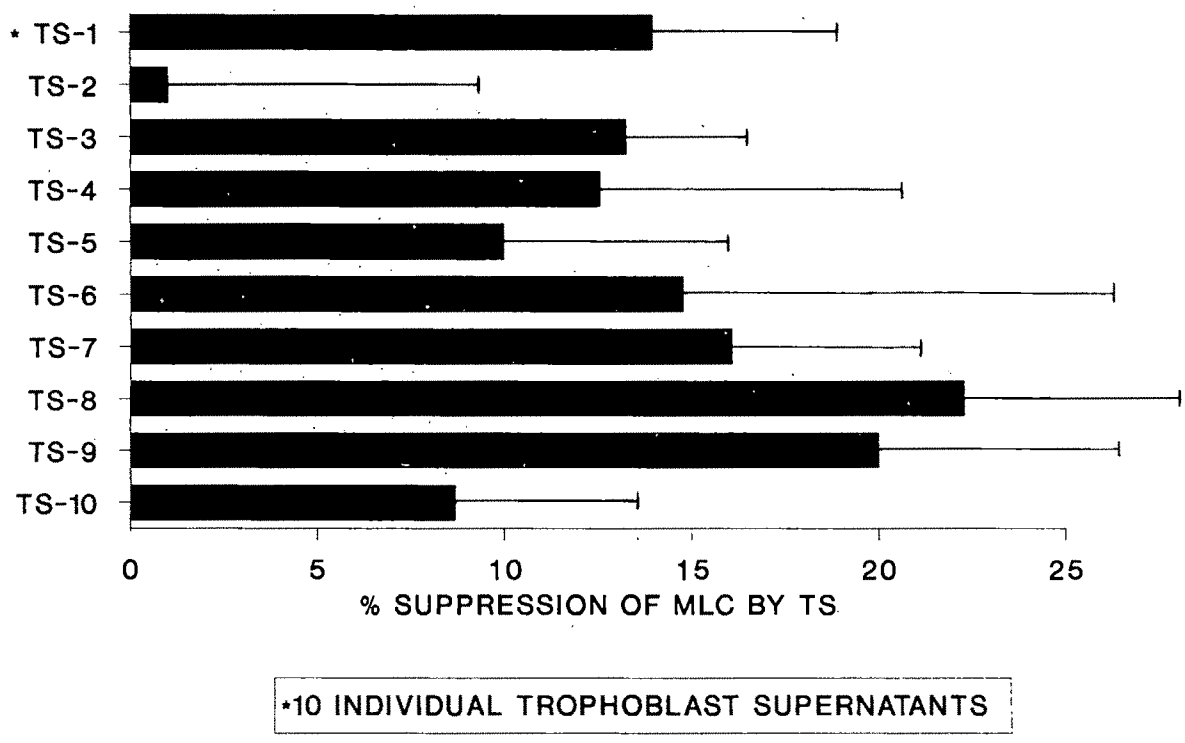


Fig. 3. Suppression of mixed lymphocyte culture *(MLC)* by 25% concentration of trophoblast supernatant *(TS)*. Means and corresponding SEs shown.

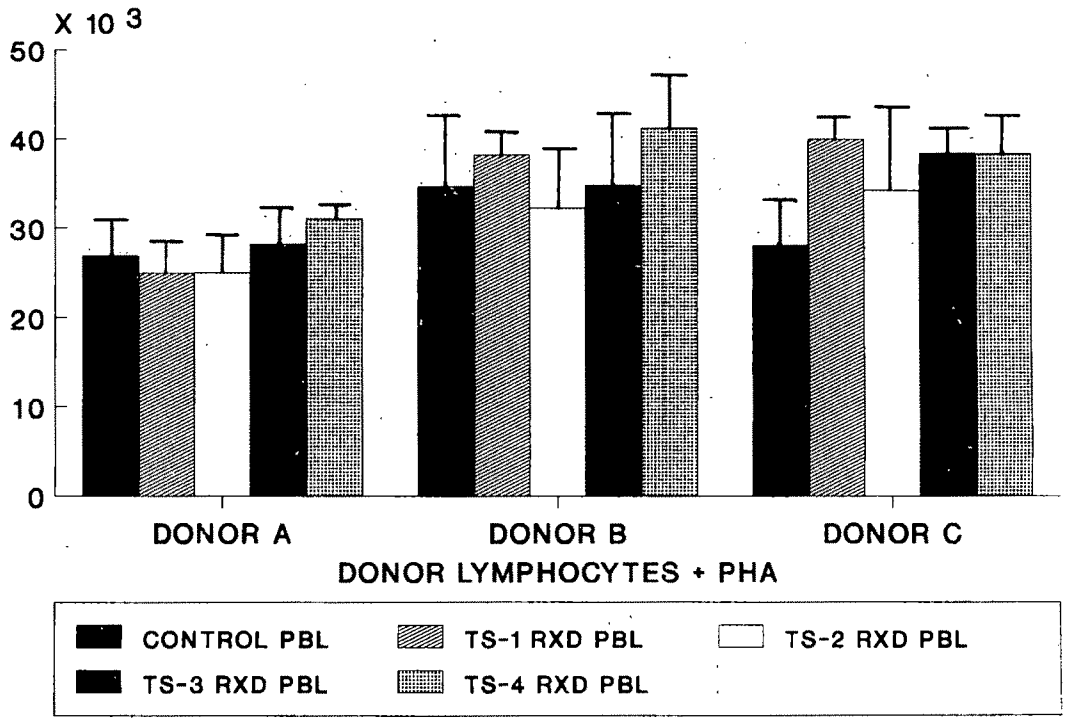


Fig. 4. T-suppressor–inducer assay: pre-treated mononuclear cells (with control media or trophoblast supernatant), then incubated with three sets of peripheral blood lymphocytes *(donors A, B, and C)* and phytohemagglutinin (PHA). No functional evidence of suppressor T-cell induction by trophoblast supernatant was observed in this experiment (or in experiments with four additional trophoblast supernatant samples; data not shown).

Table II. Exposure of mononuclear cells to a trophoblast supernatant* for 72 hours: observed changes in the proportion of mononuclear cell subpopulations

	% of total mononuclear cells		
		Postincubation	
Antibody-labeled† T-cell subpopulations	*Preincubation*	*Control (media only)*	*Experimental (media + trophoblast supernatant)*
T3 + (total T cells)	50.1	68.9	63.5
T4 + (helper T cells)	32.6	50.2	49.8
T8 + (suppressor T cells)	20.2	31.2	32.5
2H4 + (suppressor-inducer T cells)	36.1	53.9	55.5
4B4 + (helper-inducer T cells)	20.9	51.5	52.2

*The data shown represent one of the eight trophoblast supernatant samples tested in the suppressor T-cell inducer assay (Fig. 4). Similar results were obtained by incubating the other trophoblast supernatant samples with mononuclear cells from additional donors (results not shown).

†The letter-numeral combinations listed represent the monoclonal antibodies used to identify mononuclear cell subsets.

sistently suppressed mononuclear cell transformation. The ability of the first-trimester trophoblast to inhibit maternal T-cell proliferation by a paracrine mechanism may directly contribute to fetal survival. Many factors produced by the fetoplacental compartment have been reported to be immunosuppressive including chorionic gonadotropin, placental lactogen, pregnancy-associated serum protein A, and others.[16-18] However, before attempting to identify which factor(s) is responsible for local immunoregulation, the appropriateness of the trophoblast source chosen for study should be considered. By using villi retrieved during the first trimester from karyotypically normal, uncomplicated term pregnancies, and by processing these specimens

to exclude nontrophoblast cells, we believe that our in vitro observations closely resemble the paracrine function of the human trophoblast.

The immunosuppression we observed appeared to be directly related to trophoblast supernatant in a dose-dependent fashion. Unlike other studies that have implicated suppressor T-cell induction by trophoblast supernatant as the mechanism for mononuclear cell suppression, we did not confirm this phenomenon. It may be that the relatively short incubation time (72 hours) precluded the detection of suppressor T-cell subpopulations because there was insufficient time for their development. However, Sanyal et al.[10] were able to identify suppressor cell induction with similar techniques. An analysis of their data reveals that only six of the 11 trophoblast supernatant samples tested appeared to induce suppressor T-cell differentiation; a finding that we would interpret as inconclusive.

Future studies should focus on identification of the soluble factor(s) in trophoblast supernatant with immunosuppressive capability. Theoretically, a deficiency of such factors early in pregnancy might be pathologic (e.g., increase the likelihood of miscarriage or predispose a patient to recurrent pregnancy loss). Because chromosomally abnormal gestations exhibit an exaggerated rate of first-trimester fetal wastage, study of aneuploid supernatants identified at the time of chorionic villus sampling might provide a meaningful contrast to the experimental observations associated with normal pregnancies.

We thank Kris Fischer for her invaluable technical assistance in performance of the laboratory assays.

REFERENCES

1. Sutherland CA, Naiem M, Mason DY, Redman CWG, Stirrat GM. The expression of MHC antigens by human chorionic villi. J Reprod Immunol 1981;3:323-6.
2. Johnjson PM, Stern PL. Antigen expression at the maternal-fetal interfaces. In: Cinader B, Miller RG, eds. Progress in immunology. Orlando, Florida: Academic Press, 1986; VI:1056-8.
3. Kouttab WN, Fowler AK, Strichland JG, Hellman A. Suppression of in vitro lymphocyte stimulation in mice by uterine and placental extracts. J Immunol 1976;117:1644-50.
4. Chaouat G, Chaffaux S. Placental products induce suppressor cells of graft vs host reaction. Am J Reprod Immunol 1984;6:107-14.
5. Rocklin RE, Kitzmiller JL, Kaye MD. Immunobiology of the maternal-fetal relationship. Ann Rev Med 1979;30: 375-81.
6. Billington WD. Immunological aspects of implantation and fetal survival: the central role of trophoblast. Curr Top Dev Biol 1987;23:209-31.
7. Rocklin RE, Kitzmiller JL, Garvoy MR. Further characterization of an immunological blocking factor that develops during pregnancy. Clin Immunol Immunopathol 1982;22:305-15.
8. Adcock EW, Teasdale F, August CS, et al. Human chorionic gonadotropin: its possible role in maternal lymphocyte suppression. Science 1973;181:845.
9. Yagel S, Parhar RS, Lala PK. Trophic effects of first-trimester human trophoblasts and human chorionic gonadotropin on lymphocyte proliferation. Am J Obstet Gynecol 1989;160:946-53.
10. Sanyal MK, Brami CJ, Bischof P, et al. Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester. Am J Obstet Gynecol 1989;161:446-53.
11. Bobe P, Kanellopoulos-Langevin C, Bleux C, Voisin GA. Modulation of mouse anti-SRBC responses by placental extracts. II. Antigen specificity and role of B and T cell populations by two distinct placental fractions. J Immunol 1981;11:228-36.
12. Silver RK, MacGregor SN, Sholl JS, Elesh RH, Beaird JA, Waldee JK. Initiating a chorionic villus sampling program: reliance upon placental location as the determinant for the route of sampling. J Reprod Med 1990;35:964-8.
13. Simoni G, Brambati B, Danesino C, et al. Efficient direct chromosome analysis and enzyme determinations from chorionic villus samples in the first trimester of pregnancy. Hum Genetics 1983;63:349.
14. Niazi M, Coleman DV, Loeffler FE. Trophoblast sampling in early pregnancy. Br J Obstet Gynaecol 1981;88:1081.
15. Ledbetter DH, Martin AO, Verlinsky Y, et al. Cytogenetic results of chorionic villus sampling: high success rate and diagnostic accuracy in the United States collaborative study. Am J Obstet Gynecol 1990;162:495-501.
16. Contractor SF, Davies H. Effect of human chorionic somatomammotropin and human chorionic gonadotropin on phytohemagglutinin-induced lymphocyte transformation. Nature New Biol 1973;243:284-6.
17. Bischof P, Lauber K, deWustemberger B, Girard JP. Inhibition of lymphocyte transformation of pregnancy-associated plasma protein-A (PAPP-A). J Clin Lab Immunol 1982;7:F61-5.
18. Siiteri PK, Stites DP. Immunologic and endocrine interrelationships in pregnancy. Biol Reprod 1982;26:1-14.

Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies

David G. Mutch, MD, L. Stewart Massad, MD, Ming-Shian Kao, MD, and John Leslie Collins, PhD

St. Louis, Missouri

Four human cell lines derived from cervical carcinomas (ME-180, SiHa, HT-3, and MS751) and three human cell lines derived from ovarian carcinomas (SK-OV-3, Caov-3, and NIH:OVCAR-3) were analyzed in vitro to determine the effect of recombinant interferon-γ and recombinant human tumor necrosis factor-α on cell growth and survival. The effects of interferon-γ, tumor necrosis factor-α, and both interferon-γ and tumor necrosis factor-α on cell growth were measured after 24 and 72 hours of incubation by the incorporation of chromium 51. The results of this analysis showed that all seven cell lines were resistant to the antiproliferative action of tumor necrosis factor-α, that the growth of most cell lines was inhibited by interferon-γ by 72 hours of incubation, and that after 72 hours of incubation all cell lines demonstrated a synergistic antiproliferative response to the combination of interferon-γ and tumor necrosis factor-α. However, the effects of these cytokines on cell growth were found to differ among cell lines and varied with the concentration and the duration of incubation. The growth of one cell line (Caov-3) was stimulated by both tumor necrosis factor-α and interferon-γ. These results suggest that the clinical effects of these cytokines on the growth of gynecologic cancers may be more complex than previously supposed. (Am J Obstet Gynecol 1990;163:1920-4.)

Key words: Tumor necrosis factor-α, interferon-γ, cell growth, gynecologic malignancy

Cytokines are proteins, secreted by macrophages and lymphocytes, that are known to modulate the growth of various tissues. Recent advances in recombinant deoxyribonucleic acid technology have made possible the synthesis of large quantities of cytokines in pure form, allowing study of the effects of these peptides individually on a variety of cell types. The cytokines tumor necrosis factor-α and interferon-γ have shown in vitro antiproliferative effects on cell lines derived from gynecologic malignancies.[1-8] The combination of these two cytokines often acts synergistically to inhibit cell growth in vitro.[1-5] The antiproliferative effects of tumor necrosis factor-α and interferon-γ in vitro have prompted clinical studies of these cytokines as anticancer agents. Unfortunately, few clinical responses have been noted.[9-15]

The discrepancy between the clinical and in vitro responses of gynecologic cancer cells to these cytokines suggests that the actions of tumor necrosis factor-α or interferon-γ on cancer cells may be more complex than previously supposed. To evaluate the potential variability of responses to tumor necrosis factor-α and interferon-γ, we investigated the effects of these two cytokines on the growth of seven arbitrarily selected cell lines derived from human cervical and ovarian carcinomas.

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine.
Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: John L. Collins, PhD, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine, 4911 Barnes Hospital Plaza, St. Louis, MO 63110.
6/6/24727

Material and methods

Cell lines. ME-180, SiHa, HT-3, and MS751 are independent cell lines originally derived from human cervical carcinomas. SK-OV-3, Caov-3, and NIH:OVCAR-3 are independent cell lines originally derived from human ovarian carcinomas. All cell lines were obtained from the American Type Culture Collection (Rockville, Md.) and were maintained as exponentially growing cultures. All cell lines except HT-3 and NIH:OVCAR-3 were grown in Dulbecco's modified Eagle's medium supplemented with 30 mg/ml L-glutamine (Sigma Chemical Co., St. Louis), 450 U/ml penicillin (Sigma), 40μg/ml streptomycin (Sigma), and 10% fetal bovine serum (HyClone Laboratories, Logan, Utah). HT-3 cells were grown in McCoy's medium containing the same supplements as Dulbecco's modified Eagle medium. NIH:OVCAR-3 cells were grown in RPMI-1640 medium containing the

same supplements as Dulbecco's modified Eagle medium with an additional 10 μg/ml recombinant human insulin (Eli Lilly & Co., Indianapolis). NIH:OVCAR-3 cells were grown at 37° C in a humidified atmosphere of 5% carbon dioxide and 95% air. All other cells were grown at 37° C in a humidified atmosphere of 10% carbon dioxide and 90% air.

Cytokines. Recombinant human interferon-γ (2.5×10^7 U/mg) was obtained from Genzyme (Boston) and was reconstituted with Dulbecco's modified Eagle medium (10^5 U/ml). Recombinant human tumor necrosis factor-α (2×10^7 U/ml, Genzyme) also was reconstituted with Dulbecco's modified Eagle medium (10^4 U/ml). Both cytokines were stored at −70° C until use, when they were diluted in the appropriate medium used for the routine growth of cells before use in the assay of cell growth.

Assay of cell growth by incorporation of chromium 51. Cells were plated at time 0 in 96-well microtiter plates at 2×10^3 cells per well in 50 μl of the medium used for the routine growth of cells and incubated under the usual growth conditions. At 24 hours, 50 μl of interferon-γ or control medium and 50 μl of tumor necrosis factor-α or control medium were added to each well. Cells then were incubated for an additional 24 to 72 hours under usual growth conditons, when media were removed for labeling. At specified times after the addition of cytokines, each well received 30 μl of ^{51}Cr (50 μCi/ml, New England Nuclear/DuPont, Boston) in RPMI-1640 medium containing 3 gm/ml bovine serum albumin (Sigma). Plates were incubated 3 hours in a humidified atmosphere of 5% carbon dioxide at 37° C. Each well was washed four times with 100 μl Dulbecco's modified Eagle medium to remove unincorporated ^{51}Cr. Cells were lysed by the addition of 150 μl of distilled water for 15 to 20 minutes. The microtiter plates were shaken to complete lysis, then centrifuged 10 minutes. From each well, 75 μl of supernatant was removed and counted in a γ-counter. Six replicate wells containing no interferon-γ or tumor necrosis factor-α were counted from each plate. The percent growth that resulted from incubation with cytokines was calculated by the following formula: Percent growth = (cpm ^{51}Cr incorporated by treated cells/cpm ^{51}Cr incorporated by control cells) × 100.

Statistical analysis. The high and low values from each group of six wells were dropped. The means of the remaining four wells were compared with Student's *t* test. The results presented are representative of multiple experiments. Synergy between tumor necrosis factor-α and interferon-γ was assumed to exist if one or both of these agents had no significant effect on cell growth and the percent growth in the presence of their combination was significantly different from that in the presence of either cytokine alone. When both agents significantly altered growth when used alone, synergy was assessed by the construction of isobolograms.[16]

Results

Effects on cell growth of 24 hours of incubation with interferon-γ and tumor necrosis factor-α. We have previously shown that ME-180, MS751, SiHa, HT-3, SK-OV-3, NIH:OVCAR-3, and Caov-3 are all resistant to lysis by tumor necrosis factor-α and interferon-γ when measured in a 24-hour ^{51}Cr release assay.[17,18] These cytokines have also been shown to either increase or decrease the growth of different cell lines in vitro.[1-8] To determine the effects of tumor necrosis factor-α and interferon-γ on cell growth, cells were incubated with various concentrations of tumor necrosis factor-α, interferon-γ, or tumor necrosis factor-α and interferon-γ. After 24 hours of incubation, cell growth was measured by the incorporation of ^{51}Cr. The growth of treated cells was expressed as a percent of untreated control cells.

As shown in Table I, tumor necrosis factor-α did not affect the growth of any of the cell lines except that of Caov-3, which was significantly increased in the presence of tumor necrosis factor-α at concentrations of 10 and 100 U/ml. Interferon-γ at a concentration of 10 and 100 U/ml also increased the growth of the cell line Caov-3. Contrary to its growth-stimulatory effect on Caov-3 cells, interferon-γ inhibited the growth of ME-180, MS751, and HT-3 cells.

As shown in Table I, the combination of tumor necrosis factor-α and interferon-γ at 100 U/ml was synergistic in inhibiting the growth of ME-180, SiHa, HT-3, and SK-OV-3 cells. Although the growth of MS751 cells was inhibited by interferon-γ alone, the combination of tumor necrosis factor-α and interferon-γ appeared to be antagonistic (i.e., the combination did not inhibit growth). The combination of tumor necrosis factor-α and interferon-γ did not increase the proliferative effects that these two cytokines elicited individually in Caov-3 cells.

Effects on cell growth of 72 hours of incubation with of tumor necrosis factor-α and interferon-γ. To determine whether periods of incubation with tumor necrosis factor-α or interferon-γ might elicit growth responses that were quantitatively or qualitatively different from those observed at 24 hours, cells were incubated with tumor necrosis factor-α, interferon-γ, or tumor necrosis factor-α and interferon-γ for 72 hours. The growth of treated cells was compared with that of untreated cells as described above. As shown in Table II, 72 hours of incubation with cytokines resulted primarily in quantitative changes in the growth of cells compared with 24 hours of incubation.

As with 24 hours of treatment, the treatment of cells with tumor necrosis factor-α for 72 hours did not sig-

Table I. Effects of 24 hours of exposure to interferon-γ, tumor necrosis factor-α, and their combination on the growth of cell lines derived from gynecologic malignancies

	Cell growth (percentage of control)								
	Tumor necrosis factor-α (u/ml)			*Interferon-γ (u/ml)*			*Tumor necrosis factor-α and interferon-γ (u/ml)*		
Cell line	*1*	*10*	*100*	*1*	*10*	*100*	*1*	*10*	*100*
ME-180	98	116	94	104	77	69	90	60*†	13*†
MS751	93	95	101	76*	92	80*	94	105	97
SiHa	108	106	103	101	98	88	100	95	78*†
HT-3	87	96	100	91	82*	76*	100	94	83*†
SK-OV-3	81	91	88	92	93	81	79	85	74*†
NIH:OVCAR-3	101	119	104	81	88	83	95	94	83
Caov-3	152	228*	205*	123	150*	136*	167*	148*	126

*Significantly different from control, $p < 0.01$.

†Significantly different both from cells exposed to tumor necrosis factor-α and from cells exposed to interferon-γ at equal cytokine concentration, $p < 0.05$.

Table II. Effects of 72 hours of exposure to interferon-γ, tumor necrosis factor-α, and their combination on the growth of cell lines derived from gynecologic malignancies

	Cell growth (percentage of control)								
	Tumor necrosis factor-α (u/ml)			*Interferon-γ (u/ml)*			*Tumor necrosis factor-α and interferon-γ (u/ml)*		
Cell line	*1*	*10*	*100*	*1*	*10*	*100*	*1*	*10*	*100*
ME-180	101	120*	100	80	64*	34*	88	59	4*†
MS751	93	112	94	98	105	77*	83	95	64*†
HT-3	94	110	104	101	83*	68*	119*†	95	56*†
SiHa	103	103	89	97	103	73*	108	74*†	34*†
SK-OV-3	94	109	87	110	81	39*	119	76*	27*†
NIH:OVCAR-3	115	137*	108	95	93	73*	81	111*	46*†
Caov-3	165*	257*	183*	130	150	100	189*	154*	68*†

*Significantly different from control, $p < 0.01$.

†Significantly different both from cells exposed to tumor necrosis factor-α and from cells exposed to interferon-γ at the same concentration, $p < 0.05$.

nificantly inhibit the growth of any cell line, and it accelerated the growth of Caov-3 cells (Table II). Whereas treatment of NIH:OVCAR-3 cells with tumor necrosis factor-α for 24 hours had no effect, treatment for 72 hours with 10 U/ml increased the growth of these cells (Table II). The magnitude of the response of cells to interferon-γ was increased by treatment for 72 hours compared with 24 hours. Thus, extending the duration of treatment with interferon-γ from 24 to 72 hours resulted in greater growth inhibition of the cell lines ME-180, MS751, SiHa, and HT-3. In addition, the growth of two cell lines (SK-OV-3 and NIH:OVCAR-3) that was not affected by 24 hours of exposure to interferon-γ was significantly decreased when treated with 100 U/ml for 72 hours (Table II). Although treatment with interferon-γ for 24 hours increased the growth of Caov-3 cells, this response did not occur when the duration of incubation was increased to 72 hours (Table II).

As shown in Table II, synergistic growth inhibition by the combination of interferon-γ and tumor necrosis factor-α was seen in all cell lines when cells were treated with 100 U/ml of tumor necrosis factor-α and 100 U/ml of interferon-γ for 72 hours. This was true even for Caov-3 cells, which continued to show significant growth stimulation at lower concentrations (i.e., at 1 and 10 U/ml) of tumor necrosis factor-α and interferon-γ.

Although the effects of tumor necrosis factor-α, interferon-γ, or tumor necrosis factor-α and interferon-γ on cell growth were found to differ among the seven cell lines (Tables I and II), these differences were not related to inherent differences in the growth rates of the cell lines. The doubling times of all cell lines, except Caov-3, varied from 21 to 34 hours; for some cell lines that differ greatly in their response to these cytokines, there was no difference in their doubling times (data not shown).

Comment

Several authors have reported the effects of tumor necrosis factor-α and interferon-γ on the in vitro

growth of cell lines derived from cervical and ovarian malignancies.[1-8] These reports have emphasized the inhibitory actions of these cytokines, but the results of early clinical trials with these cytokines in the treatment of cancer have yielded few responses.[9-15] Our findings suggest several possible explanations for this in vivo resistance to tumor necrosis factor-α and interferon-γ.

The results presented in our report show that the antiproliferative effects of tumor necrosis factor-α and interferon-γ depend on the duration of exposure of cells to these cytokines. After 24 hours of incubation, only two of seven cell lines showed inhibition of growth by tumor necrosis factor-α (Table I). Interferon-γ inhibited the growth of five of seven cell lines at 24 hours, but the magnitude of inhibition did not become pronounced until cells were incubated with interferon-γ for 72 hours (Table II). Phase I clinical trials have shown that although significant serum concentrations of tumor necrosis factor-α and interferon-γ are achieved shortly after their administration, the levels of these cytokines decline rapidly and are rarely measurable in serum after 24 hours.[9-15] Therefore clinical efficacy may require sustained administration.

In addition to the duration of exposure to tumor necrosis factor-α and interferon-γ, our results also suggest that growth inhibition may be dose dependent. In the cell lines tested, growth inhibition rarely occurred at cytokine concentrations of 1 to 10 U/ml and only became significant at 100 U/ml. The majority of reports on the in vitro antiproliferative effects of these agents have used cytokine concentrations >10,000 U/ml.[1-8] Clinical studies have shown that levels of tumor necrosis factor-α and interferon-γ that can be sustained for several hours rarely exceed 100 U/ml.[9-14] If these cytokines only inhibit cell growth in vivo when their concentrations approximate 100 U/ml, then clinical control of tumors may require the development of techniques for the regional delivery of higher cytokine concentrations.

To date, most studies of the growth effects of tumor necrosis factor-α and interferon-γ on human cancer cells have reported on only a small number of cervical and ovarian cell lines.[1-8] Our data, based on a series of seven arbitrarily selected cell lines derived from human cervical and ovarian carcinomas, show that there is considerable heterogeneity in the responses of these cell lines to tumor necrosis factor-α and interferon-γ (Tables I and II). We found that most cell lines are resistant to growth inhibition by tumor necrosis factor-α at pharmacologic concentrations and that cell lines vary substantially in their sensitivity to interferon-γ. Caov-3 cells actually grew more rapidly in the presence of either tumor necrosis factor-α or interferon-γ. Clinically, the incidence of resistance to growth inhibition by tumor necrosis factor-α may be high, and some tumors may respond to exposure to tumor necrosis factor-α or interferon-γ with accelerated growth, as the cell line Caov-3 did in this study.

Finally, it should be noted that growth inhibition and cell lysis are not synonymous. Many assays that measure the effects of cytokines or other anticancer agents on cells compare the relative numbers of treated and untreated cells after some period of incubation. If the number of treated cells is less than the number of untreated cells, it cannot be known whether the decrease in the number of treated cells is a result of cytostasis or cytolysis. This is true regardless of how cells are enumerated and is particularly problematic with extended periods of treatment (i.e., when the period of incubation is sufficient to permit an increase in the number of untreated cells). We have shown previously that exposure of these cells to tumor necrosis factor-α or interferon-γ for up to 24 hours does not result in lysis,[17,18] and the assay technique used in this and most other studies cannot distinguish cytolysis from cytostasis.[1-3, 5-8] If in vivo, as in vitro, the effects of tumor necrosis factor-α and interferon-γ are only cytostatic, slow-growing tumors may appear unresponsive to these agents. Furthermore, the cytostatic effects of these agents may not persist after the cytokines have been cleared from the circulation, and tumors, although briefly growth inhibited, may then resume growth.

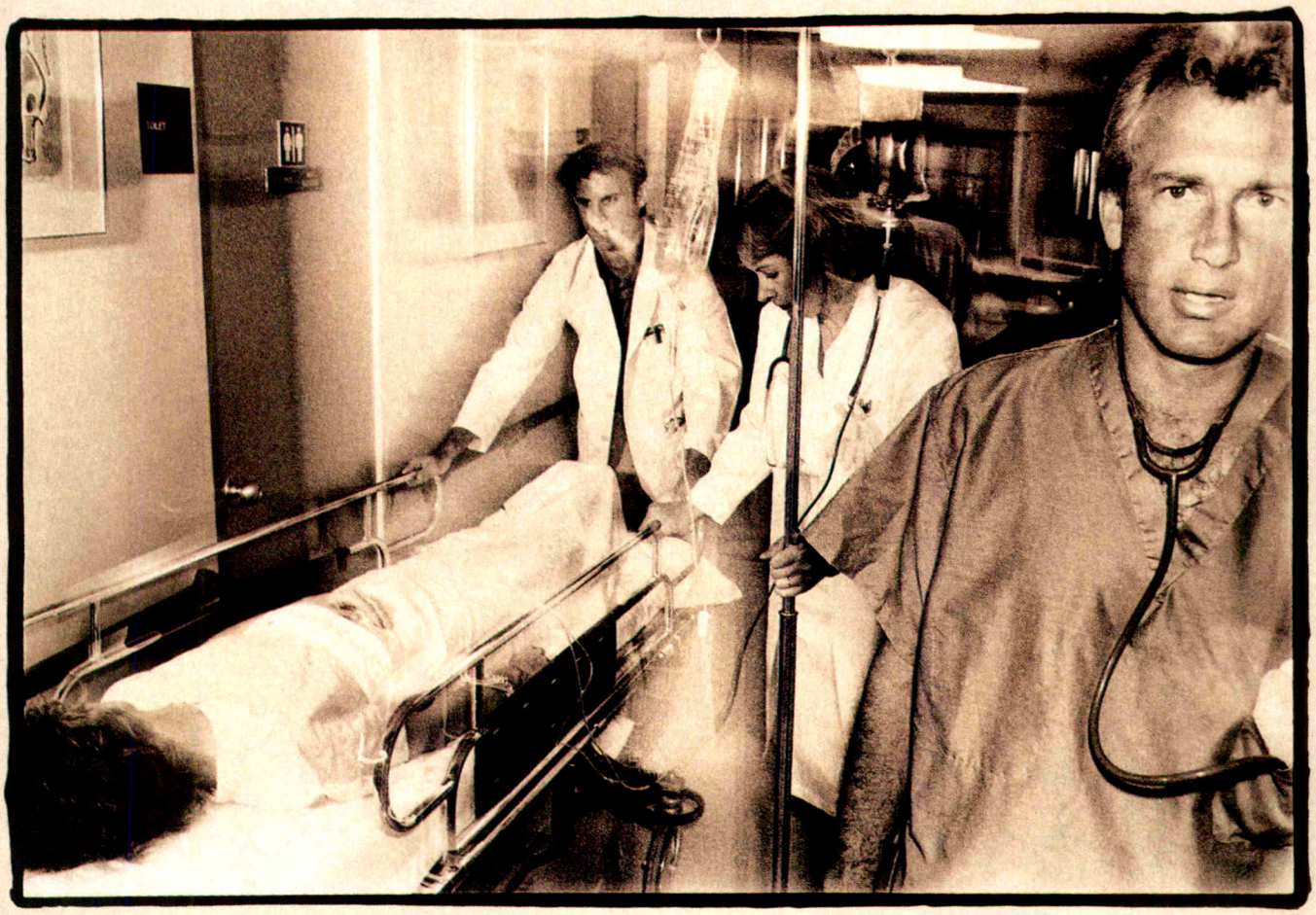
Our data confirm several reports that have noted the antiproliferative synergy of the combination of interferon-γ and tumor necrosis factor-α.[1-5] Others have proposed that such synergy may result from interferon–induced upregulation of cell surface receptors for tumor necrosis factor-α,[19] although the number of surface receptors specific for tumor necrosis factor-α does not correlate with sensitivity to tumor necrosis factor-α.[20] The results presented herein show that the addition of interferon-γ can inhibit cell growth, apparently independently of the addition of tumor necrosis factor-α. A number of cell lines of diverse histologic type have been shown to secrete tumor necrosis factor-α constitutively.[7, 21] In addition, interferon-γ has been shown to stimulate the production of tumor necrosis factor-α in monocytes.[22] To rule out the possibility that the addition of interferon-γ induces the secretion of tumor necrosis factor-α, supernatant fluids from ME-180 cells incubated with and without interferon-γ were analyzed for the presence of tumor necrosis factor-α.[23] The results of this analysis showed that ME-180 cells do not secrete tumor necrosis factor-α into the media even when interferon-γ was added (data not shown). It thus seems unlikely that interferon-γ inhibits cell growth through a mechanism involving tumor necrosis factor-α.

The results of this study suggest that the mechanism of the observed synergistic antiproliferative effect of tumor necrosis factor-α and interferon-γ may be more

complex than the simple increase in the number of tumor necrosis factor-α receptors or induction of tumor necrosis factor-α secretion by interferon-γ. Regardless of the mechanism of synergy between tumor necrosis factor-α and interferon-γ in inhibiting the growth of carcinoma cell lines, our results suggest that a combination therapy with tumor necrosis factor-α and interferon-γ would improve the efficacy of these cytokines as anticancer agents.

REFERENCES

1. Fransen L, van der Heyden J, Ruysschaert R, Fiers W. Recombinant tumor necrosis factor: its effect and its synergism with interferon-γ on a variety of normal and transformed human cell lines. Eur J Cancer Clin Oncol 1986;22:419-26.
2. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA, Shepard HM. Recombinant human tumor necrosis factor-α: effects on proliferation of normal and transformed cells in vitro. Science 1985;230:943-5.
3. Mori H, Kondoh H, Tamaya T. Synergistic effects of interferon-γ and tumor necrosis factor against proliferation of gynecologic tumor cell lines. Gynecol Oncol 1989;35:209-14.
4. Taguchi T, Abe S, Nakano K, Matsui Y, Sohmura Y. Synergistic enhancement of the antitumor activity of recombinant human TNF-α by recombinant human IFN-γ. Cancer Detect Prev 1988;12:105-14.
5. Salmon SE, Young L, Scuderi P, Clark B. Antineoplastic effects of tumor necrosis factor alpha alone and in combination with gamma-interferon on tumor biopsies in clonogenic assay. J Clin Oncol 1987;5:1816-21.
6. Jacobs AJ, Dawoud L, Kovacs Z, Ratliff TL. Inhibition of proliferation of lines derived from human cervical carcinomas by cytotoxic drugs and by recombinant interferons. Gynecol Oncol 1989;32:31-6.
7. Spriggs DR, Imamura K, Rodriguez C, Sariban E, Kufe DW. Tumor necrosis factor expression in human epithelial tumor cell lines. J Clin Invest 1988;88:455-60.
8. Marth C, Helmberg M, Mayer I, Fuith LC, Daxenbichler G, Dapunt O. Effects of biological response modifiers on ovarian carcinoma cell lines. Anticancer Res 1989;9:461-8.
9. Feinberg B, Kurzrock R, Talpaz M, Blick M, Saks S, Gutterman JU. A phase I trial of intravenously-administered recombinant tumor necrosis factor-alpha in cancer patients. J Clin Oncol 1988;6:1328-34.
10. Chapman PB, Lester TJ, Casper ES, et al. Clinical pharmacology of recombinant human tumor necrosis factor in patients with advanced cancer. J Clin Oncol 1987;5:1942-51.
11. Demetri GD, Spriggs DR, Sherman ML, Arthur KA, Imamura K, Kufe DW. A phase I trial of recombinant human tumor necrosis factor and interferon-gamma: effects of combination cytokine administration in vivo. J Clin Oncol 1989;7:1545-53.
12. Foon KA, Sherwin SA, Abrams PG, et al. A phase I trial of recombinant gamma interferon in patients with cancer. Cancer Immunol Immunother 1985;20:193-7.
13. van der Burg M, Edelstein M, Gerlis L, Lang C-M, Hirschl M, Dawson A. Recombinant interferon-γ (Immuneron): results of a phase I trial in patients with cancer. J Biol Response Mod 1985;4:264-72.
14. Kurzrock R, Rosenblum MG, Sherwin SA, et al. Pharmacokinetics, single-dose tolerance, and biological activity of recombinant γ-interferon in cancer patients. Cancer Res 1985;45:2866-72.
15. Welander CE, Homesley HD, Reich SD, Levin EA. A phase II study of the efficacy of recombinant interferon gamma in relapsing ovarian adenocarcinoma. Am J Clin Oncol 1988;11:465-9.
16. Berenbaum MC. Criteria for analyzing interactions between biologically active agents. Adv Cancer Res 1981;35:269-9.
17. Powell CB, Mutch DG, Massad LS, Kao MS, Collins JL. Common expression of a tumor necrosis factor resistance mechanism among gynecologic malignancies. Cancer Immunol Immunother [In press].
18. Massad LS, Mutch DG, Powell CB, Kao MS, Collins JL. Expression of a resistance mechanism in ovarian and cervical carcinoma cells prevents their lysis by γ-interferon. Can Res 1990;50:4923-8.
19. Tsujimoto M, Vilcek J. Tumor necrosis factor receptors in HeLa cells and their regulation by interferon-γ. J Biol Chem 1986;261:5384-8.
20. Aggarwal BB, Eessalu TE. Induction of receptors for tumor necrosis factor-α by interferons is not a major mechanism for their synergistic cytotoxic response. J Biol Chem 1987;262:10000-07.
21. Rubin BY, Anderson SL, Sullivan SA, Williamson BD, Carswell EA, Old LJ. Nonhematopoietic cells selected for resistance to tumor necrosis factor produce tumor necrosis factor. J Exp Med 1986;164:1350-5.
22. Scuderi P, Sterling KE, Raitano AB, Grogan TM, Rippe RA. Recombinant interferon-γ stimulates the production of human tumor necrosis factor in vitro. J Interferon Res 1987;7:155-64.
23. Patek PQ, Lin Y, Collins JL. Natural cytotoxic cells and tumor necrosis factor activate similar lytic mechanisms. J Immunol 1987;138:1641-6.



"It's still like walking the high wire, but now I have a safety net."

More confidence in presumptive antibiotic therapy. Prescribing UNASYN presumptively is a step you can take with confidence. UNASYN covers gram-positives (even enterococci), prevalent gram-negatives, and anaerobes: The right combination for most patients with common mixed infections.*

And UNASYN minimizes safety concerns with a reliable penicillin-class profile.[1] (However, UNASYN is contraindicated in penicillin-allergic patients.)

Expect strong support from UNASYN in the mixed infections* you face every day.

1.5 or 3g q6h

Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

An antibiotic for every day

ROERIG Pfizer
A division of Pfizer Pharmaceuticals

*Due to susceptible strains of indicated organisms.

Please see brief summary of prescribing information on adjacent page.

© 1989, Pfizer Inc.

CURRENT PROBLEMS IN OBSTETRICS, GYNECOLOGY AND FERTILITY

Editor-in-Chief: **Robert L. Barbieri, MD**
State University of New York at Stony Brook

Current Problems in Obstetrics, Gynecology and Fertility brings you the authoritative, practical information you need to stay current in your specialty and to provide the best possible care to patients.

- Each bimonthly issue provides in-depth, monograph-length coverage (40-60 pages) of a clinical topic of vital interest to practitioners today.
- Topics are selected for relevance to your daily practice: preconceptual counseling, infertility, endocrine problems, high-risk obstetrics, diagnosis and treatment of primary and secondary amenorrhea, pediatric and adolescent gynecology, and more.
- Authors are selected for their expertise; you'll benefit from their balanced perspective on the topic and their wealth of clinical experience.
- Each article is extensively referenced, saving you hours of library research and serving as a long-term, practical resource.

Subscribe today by returning the attached order form or by calling our toll-free number!

Mosby Year Book

Yes! Please begin my subscription to ***Current Problems in Obstetrics, Gynecology and Fertility*** (6 issues per year).

1991 Rates	U.S.A.	Canada	International
☐ Individual	$60.00	$67.00	$85.00*
☐ Student, resident	39.00	46.00	64.00*
☐ Institutional	85.00	92.00	110.00*

* Exclusive of Japan and India. Contact Publisher for rate and agent information.

Individual and student/resident subscriptions must be in the name of, billed to, and paid for by the individual. Please allow 6-8 weeks for receipt of first issue in U.S. Price of international subscriptions includes surface-mail postage.

☐ Send me a custom binder for $20.00. (Holds 12 issues.)

Method of Payment (please check one)

We'll send an extra issue free if payment accompanies your order!

☐ Check (U.S. funds, payable to *Current Problems in Obstetrics, Gynecology and Fertility*)

☐ MasterCard ☐ MC International ☐ VISA ☐ Bill me

Credit card # _____ Exp. date _____

Signature _____

Name _____

Institution _____

Address _____

City _____ State _____ ZIP _____

Country _____ Specialty _____

Return to:

Mosby–Year Book, Inc., Journal Subscription Services,
11830 Westline Industrial Drive, St. Louis, MO 63146 U.S.A.
For faster service, phone: (314) 872-8370, ext. 7351
In the U.S., call toll-free: 1-800-325-4177, ext. 7351
Fax: (314) 432-1380

J5290FZC

Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

1.5 or 3g q6h

References:

1. Data available on request from Roerig. **2.** Aldridge KE, Sanders CV, Marier RL: Variation in the potentiation of β-lactam antibiotic activity by clavulanic acid and sulbactam against multiply antibiotic-resistant bacteria. *J Antimicrob Chemother* 1986;17:463-469. **3.** Crombleholme WR, Ohm-Smith M, Robbie MO, et al: Ampicillin/sulbactam versus metronidazole-gentamicin in the treatment of soft tissue pelvic infections. *Am J Obstet Gynecol* 1987;156(Feb):507-512. **4.** Cucharal G, Snydman D, Jacobus N, et al: Comparison of new antimicrobial agents against the *Bacteroides fragilis* group. Abstract, presented at the Annual Meeting of the American Society of Microbiology, Miami Beach, Fla, May 1988. **5.** D'Amato RF, Hochstein L, Pavia M: *In vitro* susceptibilities of aerobic and anaerobic clinical isolates to ampicillin/sulbactam. Presented as a Scientific Exhibit at the 87th Annual Meeting of the American Society of Microbiology, Atlanta, March 1987. **6.** Finegold SM: Anaerobic bacteria: Their role in infection and their management. *Postgrad Med* 1987;81(8):141-147. **7.** Jenkins SG: Susceptibility patterns of *Bacteroides fragilis* group isolates to ampicillin/sulbactam and other selected antibiotics. Presented at a symposium entitled *Ampicillin/Sulbactam: New Strategy for the Treatment of Infectious Disease*, Montreux, Switzerland, July 16-17, 1987, pp 19-20. **8.** Tally FP, Jacobus NV: Activity of sulbactam against recent isolates of aerobic and anaerobic bacteria. abstract 36. Presented at the 23rd International Congress of Antimicrobial Agents and Chemotherapy, Las Vegas, Oct 24-26, 1983 (abs). **9.** Wasilauskas B: Susceptibility of anaerobes to ampicillin-sulbactam and 6 other antimicrobial agents. abstract. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, Fla, May 8-13, 1988. **10.** Wexler HM, Harris B, Carter WT, et al: In vitro efficacy of sulbactam combined with ampicillin against anaerobic bacteria. *Antimicrob Agents Chemother* 1985;27:876-878. **11.** Ampicillin/sulbactam (Unasyn). *Med Lett Drugs Ther* 1987;29(August 28):79-81. **12.** Hemsell DL, Wendel GD, Hemsell PG: Combating beta-lactamase enzyme—principal defense mechanism of many pelvic infection pathogens. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. **13.** Senft H-H, Stiglmayer R, Eibach HW, et al: Sulbactam/ampicillin versus cefoxitin in the treatment of obstetric and gynaecological infections. *Drugs* 1986;31(suppl 2):18-21. **14.** Newton ER, Gibbs RS: Treatment of postpartum endometritis: A comparison of ampicillin/sulbactam vs. gentamicin plus clindamycin. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. **15.** Gunning J: A comparison of parenteral sulbactam/ampicillin versus clindamycin/gentamicin in the treatment of pelvic inflammatory disease. *Drugs* 1986;31(suppl 2):14-17. **16.** Stromberg BV, Reines HD, Hunt P: Comparative clinical study of sulbactam and ampicillin and clindamycin and tobramycin in infections of soft tissues. *Surg Gyn Obstet* 1986;162:575-578. **17.** Kager L, Malmborg AS, Nord CE, et al: A randomized controlled trial of ampicillin plus sulbactam vs. gentamicin plus clindamycin in the treatment of intraabdominal infections: A preliminary report. *Rev Infect Dis* 1986;8(suppl 5):S583-S588. **18.** Bruhat MA, Pouly JL, Le Boedec G, et al: Treatment of acute salpingitis with sulbactam/ampicillin: Comparison with cefoxitin. *Drugs* 1986;31(suppl 2):7-10. **19.** Faro S: Experience with ampicillin/sulbactam for OB/GYN infections. Presented at a symposium entitled *β-Lactamase Inhibitors: Progress with Ampicillin/Sulbactam*, Atlanta, December 6, 1987, p 14 (abs).

INDICATIONS AND USAGE

UNASYN is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below.

Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus*, *Escherichia coli*,* *Klebsiella* spp.* (including *K. pneumoniae**), *Proteus mirabilis*,* *Bacteroides fragilis*,* *Enterobacter* spp.,* and *Acinetobacter calcoaceticus*.*

Intra-Abdominal Infections caused by beta-lactamase producing strains of *Escherichia coli*, *Klebsiella* spp. (including *K. pneumoniae**), *Bacteroides* spp. (including *B. fragilis*), and *Enterobacter* spp.*

Gynecological Infections caused by beta-lactamase producing strains of *Escherichia coli*,* and *Bacteroides* spp.* (including *B. fragilis**).

*Efficacy for this organism in this organ system was studied in fewer than 10 infections.

While UNASYN* (ampicillin sodium/sulbactam sodium) is indicated only for the conditions listed above, infections caused by ampicillin-susceptible organisms are also amenable to treatment with UNASYN due to its ampicillin content. Therefore, mixed infections caused by ampicillin-susceptible organisms and beta-lactamase producing organisms susceptible to UNASYN should not require the addition of another antibiotic.

CONTRAINDICATIONS

The use of UNASYN is contraindicated in individuals with a history of hypersensitivity reactions to any of the penicillins.

WARNINGS

SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE APT TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR HYPERSENSITIVITY REACTIONS TO MULTIPLE ALLERGENS. THERE HAVE BEEN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE THERAPY WITH A PENICILLIN, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS, AND OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, UNASYN SHOULD BE DISCONTINUED AND THE APPROPRIATE THERAPY INSTITUTED.

SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS, AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

PRECAUTIONS

General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.

Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.

Drug/Laboratory Test Interactions: Administration of UNASYN will result in high urine concentrations of ampicillin. High urine concentrations of ampicillin may result in false positive reactions when testing for the presence of glucose in urine using Clinitest™, Benedict's Solution or Fehling's Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix™ or Testape™) be used. Following administration of ampicillin to pregnant women, a transient decrease in plasma concentration of total conjugated estriol, estriol-glucuronide, conjugated estrone, and estradiol has been noted. This effect may also occur with UNASYN.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential.

Pregnancy

Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up to ten (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed. (See—Drug/Laboratory Test Interactions.)

Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it is not known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.

Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, caution should be exercised when UNASYN is administered to a nursing woman.

Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under the age of 1.2.

ADVERSE REACTIONS

UNASYN is generally well tolerated. The following adverse reactions have been reported:

Local Adverse Reactions

Pain at IM injection site—16% Pain at IV injection site—3%
Thrombophlebitis—3%

Systemic Adverse Reactions

The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than 2% of the patients.

Additional systemic reactions reported in less than 1% of the patients were: itching, nausea, vomiting, candidiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine retention, dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis and mucosal bleeding.

Adverse Laboratory Changes

Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:

Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.

Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and increased lymphocytes, monocytes, basophils, eosinophils, and platelets.

Blood Chemistry: Decreased serum albumin and total proteins.

Renal: Increased BUN and creatinine.

Urinalysis: Presence of RBC's and hyaline casts in urine.

The following adverse reactions have been reported with ampicillin-class antibiotics and can also occur with UNASYN:

Gastrointestinal: Gastritis, stomatitis, black hairy tongue, enterocolitis and pseudomembranous colitis.

Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatitis have been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicillin (see WARNINGS).

Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has been reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena.

ROERIG Pfizer
A division of Pfizer Pharmaceuticals

38A



"I used to think only <u>two</u> things in life were certain."

Nothing comes closer to 100% activity against anaerobes in vitro. It's a fact. Studies have proved no other antibiotic superior to UNASYN in overall antianaerobic activity.[1-10] Not clindamycin. Not metronidazole. Nothing.

And that's nothing to overlook—given the importance of anaerobes in today's mixed infections.

Though clinical results may not always mirror those obtained in vitro, there's a world of confidence in choosing UNASYN for presumptive therapy of the mixed infections* you see every day.

1.5 or 3g q6h

Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

An antibiotic for every day

ROERIG Pfizer
A division of Pfizer Pharmaceuticals

*Due to susceptible strains of indicated organisms.

Please see brief summary of prescribing information on adjacent page.

© 1989, Pfizer Inc.

PERSONALIZED LIBRARY CASES

Protect your personal copies of
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY
in custom made, titled library cases.

Constructed of durable, reinforced binder's board covered in a rich simulated leather, each is designed to hold an entire year's issues. The Journal name is stamped in gold leaf, making the cases attractive additions to any decor, whether on your bookshelf, desk or any location in your home or office.

Only $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.). U.S. funds only.

To charge your order using American Express, Visa, MasterCard or Diners Club, call toll-free: 1-800-972-5858.

Please send me _____ library cases for
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY
at $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.).

Name _____
(please print legibly or type)

Address _____
(no P.O. boxes, please)

City / State / Zip _____

PA residents add 6% sales tax

Jesse Jones Industries
499 East Erie Avenue
Philadelphia, PA 19134
Department Code: Mosby

THE UNIVERSITY OF CHICAGO

LASER SURGERY COURSE FOR PHYSICIANS

March 21-22, 1991
The University of Chicago Medical Center

Classroom instruction in laser physics, laser safety and clinical applications. Hands-on training for Nd:YAG, KTP, Argon, CO_2. Simultaneous sessions for nurses & technicians.

Approved by The American Society for Laser Medicine and Surgery

For further information, contact:
The University of Chicago, Center for Continuing Medical Education, 5841 South Maryland, Box 139, Chicago, IL 60637; (312) 702-1056

Unasyn® IM IV
(ampicillin sodium/sulbactam sodium)

1.5 or 3g q6h

References:

1. Data available on request from Roerig. **2.** Aldridge KE, Sanders CV, Marier RL: Variation in the potentiation of β-lactam antibiotic activity by clavulanic acid and sulbactam against multiply antibiotic-resistant bacteria. *J Antimicrob Chemother* 1986;17:463-469. **3.** Crombleholme WR, Ohm-Smith M, Robbie MO, et al: Ampicillin/sulbactam versus metronidazole-gentamicin in the treatment of soft tissue pelvic infections. *Am J Obstet Gynecol* 1987;156(Feb):507-512. **4.** Cucharal G, Snydman D, Jacobus N, et al: Comparison of new antimicrobial agents against the *Bacteroides fragilis* group. Abstract, presented at the Annual Meeting of the American Society of Microbiology, Miami Beach, Fla; May 1988. **5.** D'Amato RF, Hochstein L, Pavia M: *In vitro* susceptibilities of aerobic and anaerobic clinical isolates to ampicillin/sulbactam. Presented as a Scientific Exhibit at the 87th Annual Meeting of the American Society of Microbiology, Atlanta, March 1987. **6.** Finegold SM: Anaerobic bacteria. Their role in infection and their management. *Postgrad Med* 1987;81(8):141-147. **7.** Jenkins SG: Susceptibility patterns of *Bacteroides fragilis* group isolates to ampicillin/sulbactam and other selected antibiotics. Presented at a symposium entitled *Ampicillin/Sulbactam: New Strategy for the Treatment of Infectious Disease*. Montreux, Switzerland; July 16-17, 1987; pp 19-20. **8.** Tally FP, Jacobus NV: Activity of sulbactam against recent isolates of aerobic and anaerobic bacteria; abstract 36. Presented at the 23rd International Congress of Antimicrobial Agents and Chemotherapy, Las Vegas, Oct 24-26, 1983 (abs). **9.** Wasilauskas B: Susceptibility of anaerobes to ampicillin-sulbactam and 6 other antimicrobial agents; abstract. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, Fla, May 8-13, 1988. **10.** Wexler HM, Harris B, Carter WT, et al: In vitro efficacy of sulbactam combined with ampicillin against anaerobic bacteria. *Antimicrob Agents Chemother* 1985;27:876-878. **11.** Ampicillin/sulbactam (Unasyn). *Med Lett Drugs Ther* 1987;29(August 28):79-81. **12.** Hemsell DL, Wendel GD, Hemsell PG: Combating beta-lactamase enzyme—principal defense mechanism of many pelvic infection pathogens. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. **13.** Senft H-H, Stiglmayer R, Eibach HW, et al: Sulbactam/ampicillin versus cefoxitin in the treatment of obstetric and gynaecological infections. *Drugs* 1986;31(suppl 2):18-21. **14.** Newton ER, Gibbs RS: Treatment of postpartum endometritis. A comparison of ampicillin/sulbactam vs. gentamicin plus clindamycin. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. **15.** Gunning J: A comparison of parenteral sulbactam/ampicillin versus clindamycin/gentamicin in the treatment of pelvic inflammatory disease. *Drugs* 1986;31(suppl 2):14-17. **16.** Stromberg BV, Reines HD, Hunt P: Comparative clinical study of sulbactam and ampicillin and clindamycin and tobramycin in infections of soft tissues. *Surg Gyn Obstet* 1986;162:575-578. **17.** Kager L, Malmborg AS, Nord CE, et al: A randomized controlled trial of ampicillin plus sulbactam vs. gentamicin plus clindamycin in the treatment of intraabdominal infections: A preliminary report. *Rev Infect Dis* 1986;8(suppl 5):S583-S588. **18.** Bruhat MA, Pouly JL, Le Boedec G, et al: Treatment of acute salpingitis with sulbactam/ampicillin. Comparison with cefoxitin. *Drugs* 1986;31(suppl 2):7-10. **19.** Faro S: Experience with ampicillin/sulbactam for OB/GYN infections. Presented at a symposium entitled *β-Lactamase Inhibitors: Progress with Ampicillin/Sulbactam*. Atlanta, December 6, 1987; p 14 (abs).

INDICATIONS AND USAGE

UNASYN is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below.

Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus*, *Escherichia coli*,* *Klebsiella* spp.* (including *K. pneumoniae**), *Proteus mirabilis*,* *Bacteroides fragilis*,* *Enterobacter* spp.*, and *Acinetobacter calcoaceticus*.*

Intra-Abdominal Infections caused by beta-lactamase producing strains of *Escherichia coli*, *Klebsiella* spp. (including *K. pneumoniae**), *Bacteroides* spp. (including *B. fragilis*), and *Enterobacter* spp.*

Gynecological Infections caused by beta-lactamase producing strains of *Escherichia coli*,* and *Bacteroides* spp.* (including *B. fragilis**)

*Efficacy for this organism in this organ system was studied in fewer than 10 infections.

While UNASYN* (ampicillin sodium/sulbactam sodium) is indicated only for the conditions listed above, infections caused by ampicillin-susceptible organisms are also amenable to treatment with UNASYN due to its ampicillin content. Therefore, mixed infections caused by ampicillin-susceptible organisms and beta-lactamase producing organisms susceptible to UNASYN should not require the addition of another antibiotic.

CONTRAINDICATIONS

The use of UNASYN is contraindicated in individuals with a history of hypersensitivity reactions to any of the penicillins.

WARNINGS

SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE APT TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR HYPERSENSITIVITY REACTIONS TO MULTIPLE ALLERGENS. THERE HAVE BEEN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE THERAPY WITH A PENICILLIN, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS, AND OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, UNASYN SHOULD BE DISCONTINUED AND THE APPROPRIATE THERAPY INSTITUTED.

SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS, AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

PRECAUTIONS

General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN, the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.

Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.

Drug/Laboratory Test Interactions: Administration of UNASYN will result in high urine concentrations of ampicillin. High urine concentrations of ampicillin may result in false positive reactions when testing for the presence of glucose in urine using Clinitest.® Benedict's Solution or Fehling's Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix.® or Testape.®) be used. Following administration of ampicillin to pregnant women, a transient decrease in plasma concentration of total conjugated estriol, estriolglucuronide, conjugated estrone, and estradiol has been noted. This effect may also occur with UNASYN.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential.

Pregnancy

Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up to ten (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed. (See—Drug/Laboratory Test Interactions.)

Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it is not known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.

Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, caution should be exercised when UNASYN is administered to a nursing woman.

Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under the age of 12.

ADVERSE REACTIONS

UNASYN is generally well tolerated. The following adverse reactions have been reported:

Local Adverse Reactions

Pain at IM injection site—16% Pain at IV injection site—3%
Thrombophlebitis—3%

Systemic Adverse Reactions

The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than 2% of the patients.

Additional systemic reactions reported in less than 1% of the patients were: itching, nausea, vomiting, candidiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine retention, dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis and mucosal bleeding.

Adverse Laboratory Changes

Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:

Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.

Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and increased lymphocytes, monocytes, basophils, eosinophils, and platelets.

Blood Chemistry: Decreased serum albumin and total proteins.

Renal: Increased BUN and creatinine.

Urinalysis: Presence of RBC's and hyaline casts in urine.

The following adverse reactions have been reported with ampicillin-class antibiotics and can also occur with UNASYN:

Gastrointestinal: Gastritis, stomatitis, black "hairy" tongue, enterocolitis and pseudomembranous colitis.

Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatitis have been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicillin (see WARNINGS).

Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has been reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena.

ROERIG Pfizer
A division of Pfizer Pharmaceuticals
New York, New York 10017

© 1988, Pfizer Inc

INDICATIONS AND USAGE

UNASYN is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below.

Skin and Skin Structure Infections caused by beta-lactamase producing



"We need gold-standard results that don't cost a fortune."

Lower antibiotic costs without lowering your standards. UNASYN costs at least 40% less per day than clindamycin/gentamicin or cefoxitin.[11] Yet UNASYN is at least as effective in a variety of mixed infections.*[3,12-19]

And in some studies versus combination therapy, patients treated with UNASYN spent fewer days in the hospital.[3,16,17]

So, look to UNASYN for highly effective, highly affordable therapy in mixed infections* every day.

1.5 or 3g q6h

Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

An antibiotic for every day

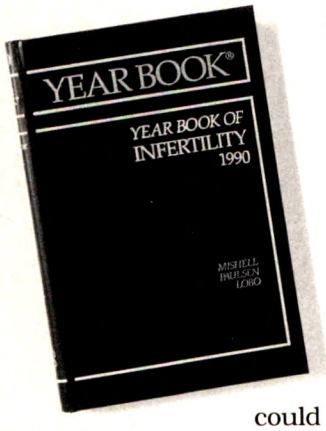
ROERIG Pfizer
A division of Pfizer Pharmaceuticals

*Due to susceptible strains of indicated organisms.

Please see brief summary of prescribing information on adjacent page.

©1989, Pfizer Inc.

Smart, current, in-depth, tough, unique, charismatic, authoritative, handy, relevant, lucid, thorough, convenient, detailed, pithy, top-notch, helpful, sagacious, indispensible, comprehensive, thought-provoking, timely, incisive, straightforward, lively, astute, invaluable, concise, fact-packed, and one-of-a-kind.



Okay. So we went a little overboard. But we could go on and on about the *Year Book of Infertility.* We could gush about how it gives you abstracts of the 310 most important articles from the past year. And we could carry on about our distinguished editors who comment on the significance and clinical application of the abstracts.

But let's be honest, you've probably seen other publishers use these same words to describe their products. And no list of adjectives can really convey a book's usefulness and value. *You've got to evaluate it for yourself.*

That's why we're offering you this no-risk, no-obligation, 30-day, free trial of the *Year Book of Infertility.* Just give us a call or drop this coupon in the mail and we'll send you a free examination copy. Put it to work for a month, and if you're not completely satisfied, just send it back and owe nothing.

We are confident, however, that you'll want to add a few adjectives of your own to this list—extraordinary, delectable, and revolutionary are still available.

❑ **Yes!**
Please send my free 30-day examination copy of the *1990 Year Book of Infertility* (20414). If I decide to keep the book I'll pay just $51.95 plus shipping and handling.

Name

Address

City, State, Zip Telephone

Mosby Year Book

Call TOLL FREE 1-800-426-4545

Or send this coupon Attn: Pat Newman, 11830 Westline Industrial Drive, St. Louis, MO 63146-9988

MCA-025

This Year Book is published annually. For the convenience of our customers, Mosby-Year Book enters each purchaser as a subscriber to future volumes and sends annual announcements approximately 2 months before publication. The new volume will be shipped upon publication unless you complete and return the announcement requesting cancellation within the time indicated. You may cancel your subscription at any time. Each new volume may be examined for 30 days, and may be returned for full credit. Return postage is guaranteed.

WE HAVE A POINT TO MAKE.



SO LITTLE CAN DO SO MUCH.

The Smart Start

OVCON® 35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Ovcon® 35 offers your patients contraception, with the lowest norethindrone dose of any combination OC. Low incidence of both androgenic side effects[1] and breakthrough bleeding.[2]

Ovcon 35 has a unique estrogen/progestogen ratio. And remember—Ovcon 35 has no generic equivalent.

Amazing how so little can do so much. Our point. Exactly.

1. Data on file, Bristol-Myers Company.
2. Gordon M, Boria M: Multiclinic study of a low-dose oral contraceptive, in *The Metabolic Effects of a Low-Estrogen/Low-Progestogen Oral Contraceptive.* New York, Biomedical Information Corp., 1981, pp 8-11.

Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.

Dedicated to Women's Health Care

Please see next page for brief summary of complete prescribing information.

C-K39-1989

© 1989, Bristol-Myers Company, Evansville, Indiana 47721 U.S.A.

OVCON® 35

(norethindrone [0.4 mg] and ethinyl estradiol [0.035 mg]) tablets

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Dose-related Risk of Thromboembolism from Oral Contraceptives: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 50 mcg or less of estrogen.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy. 8) Benign or malignant liver tumor which developed during the use of oral contraceptive or other estrogen-containing products.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34, 5/100,000; ages 35-44, 33/100,000; ages 45-49 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were age 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately.If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. (2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledemia; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy. Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstibestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) *Gallbladder Disease.* Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Headaches.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. (11) *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) *Breast-Feeding.* A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norephinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein -bound iodine (PBI), T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG, free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. 11) The active yellow tablets and the inert green tablets in the 28 day regimen of Ovcon-35 contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals. Although the overall incidence of FD&C Yellow No. 5 (tartrazine) sensitivity in the general population is low, it is frequently seen in patients who also have aspirin hypersensitivity.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (other reactions, as a general rule, are seen much less frequently or only occasionally); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; changes in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, hemolytic uremic syndrome, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erhythema nodosum, hemorrhagic eruption, vaginitis, porphyria.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Mead Johnson
LABORATORIES
A Bristol-Myers Company
Evansville, Indiana 47721
U.S.A.

C-K39-1989

© 1989, Bristol-Myers Company, Evansville, Indiana 47721 U.S.A.

Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm

Hildur Hardardottir, MD,[a] Tim H. Parmley II, MD,[a] J. Gerald Quirk, Jr., MD, PhD,[a] M. Melinda Sanders, MD,[b] Frank C. Miller, MD,[a] and Timothy J. O'Brien, PhD[a]

Little Rock, Arkansas

New murine monoclonal antibodies to a partially purified CA 125 antigen were developed and identified as M 2 and M 11. With immunohistochemical techniques, these new antibodies and OC 125 antibody were used to search for CA 125 in embryonic tissues and adult apocrine sweat glands and mammary glands. The embryonic skin, the periderm, expressed CA 125 antigen and its adult derivatives, the mammary glands and apocrine sweat glands, expressed CA 125 while in the active state of secretion. In a 6-week-old formalin-fixed and paraffin-embedded ectopic embryo specimen, antibodies M 2 and M 11 recognized CA 125 in the periderm, the notochord, the myocardium, the pericardium, the gastroenteric tract, enteric duct remnants in the umbilical cord (vitelline and allantoic ducts), the mesonephric duct, and the amnion. OC 125 staining of these formalin-fixed specimens was either very faint or absent. In a formalin-fixed and paraffin-embedded specimen of apocrine sweat glands from the axilla, antibodies M 2 and M 11 detected CA 125 antigen intracellularly in the secretory cells. Again no staining was observed with OC 125 antibody. In a frozen and acetone-fixed specimen of lactating mammary glands, antibodies M 2 and OC 125 detected CA 125 antigen intraductally. Colostrum and milk collected from 25 mothers at various stages post partum had mean CA 125 levels of 34,213 U/ml in colostrum, 1469 U/ml at 3 to 7 days, and 105 U/ml at 5 to 26 weeks. (Am J Obstet Gynecol 1990;163:1925-31.)

Key words: CA 125, periderm, apocrine glands, breast milk

In 1981 Bast and colleagues[1] identified OC 125, a monoclonal antibody that recognizes the CA 125 epitope associated with a high molecular weight glycoprotein antigen complex. Since then, the antigen has been localized immunohistochemically in many ovarian carcinomas of epithelial origin with the use of OC 125 antibody.[2-4] It has also been found in the serum of women with these tumors and has been widely used as a tumor marker in selected cases.[5-7] The antigen also has been measured in the serum of patients with endometrial,[8] pancreatic,[5] and pulmonary carcinoma[9] as well as in patients with pelvic inflammatory disease,[10] endometriosis[11-13] and in pregnancy.[14] In pregnancy it is found not only in maternal serum but also in amniotic fluid.[15, 16] With the use of immunohistochemical techniques, CA 125 has been detected intracellularly in the periderm and amnion of a 7-week human embryo.[17] After differentiation of the embryonic coelomic epithelium CA 125 is found in the pleura, pericardium, peritoneum, and in the müllerian duct derivatives: the corpus uteri, the cervix uteri, and the fallopian tube.[17, 18] In the adult, some antigen expression is maintained in these tissues.[18, 19]

Recently we developed new murine monoclonal antibodies to a preparation that contained partially purified CA 125 (unpublished data). With OC 125 and these new antibodies, M 2 and M 11, we studied immunohistochemically the tissues of a formalin-fixed, paraffin-embedded ectopic embryo of approximately 6 weeks' gestational age. Adult derivatives of the fetal periderm and their secretory products were also evaluated by both immunohistochemistry and direct immunoradiometric assay.

Material and methods

Antibody preparation. Monoclonal antibodies to antigen containing CA 125 were produced by immunizing Balb/c mice with partially purified CA 125 antigen followed by lymphocyte fusion with a mouse myeloma cell line (unpublished data). Hybrids were screened for CA 125–specific antibodies and positive cultures were cloned by dilution for hybridoma selection. Productive clones were grown in the peritoneal cavity of pristane-treated mice and monoclonal antibodies harvested from ascites. This ascites fluid was aliquotted and stored at −70° C for further use.

Tissue samples. A formalin-fixed and paraffin-embedded ectopic embryo specimen, apocrine sweat glands from the axilla, groin, and a dermoid cyst, as well as fetal membranes from a normal placenta at term

From the Departments of Obstetrics and Gynecology[a] and Pathology,[b] University of Arkansas for Medical Sciences.
Supported by National Institutes of Health Grant No. ROICA40406.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Timothy J. O'Brien, PhD, University of Arkansas for Medical Sciences, Department of Obstetrics and Gynecology, 4301 W. Markham, Slot 518, Little Rock, AR 72205.
6/6/24776

were provided by the Pathology Department at the University of Arkansas for Medical Sciences.

Sections were cut at 4 μm intervals and mounted on gelatin-coated slides. After deparaffinization in toluene (three changes, 5 minutes each) and rehydration through serial ethanol baths, tissue sections were permeabilized with a detergent (0.05% Triton-X 100, 10 minutes) and acid (0.2 mol/L hydrochloric acid, 20 minutes) before endogenous peroxidase activity was blocked with hydrogen peroxide (0.03% in 100% methanol, 30 minutes). Secondary antibody–human antigen interactions were blocked with γ-globulin (immunoglobulin G) fraction of serum from nonimmune rabbits (DAKO, Santa Barbara, Calif.) 30 minutes at room temperature. The sections were then incubated overnight at room temperature with one of the following murine antihuman antibodies: M 2 diluted 1 : 50, 1 : 100, and 1 : 500, M 11 diluted 1 : 100 and 1 : 500, and OC 125 diluted 1 : 50, 1 : 100, and 1 : 500 in phosphate buffer solution. As a negative control, mouse ascites was similarly incubated on adjacent sections at the same dilution as was the primary antibody. A subsequent 30-minute incubation with secondary antibody, rabbit antimouse, was followed by a 30-minute incubation with mouse peroxidase-antiperoxidase, both at room temperature. The chromogen used to stain immunoreactive cells was 3-amino-9-ethylcarbazole. Between steps, the sections were washed in Tris hydrochloride saline solution buffer for three washings of 5 minutes each. The secondary antibody, the mouse peroxidase-antiperoxidase, and the chromogen were all supplied by DAKO. Tissue sections were counterstained with Gill's hemotoxylin, bathed in lithium carbonate solution, and coverslipped with warm glycerol-gelatin.

Frozen lactating mammary gland tissue was supplied by the Cooperative Human Tissue Network, Birmingham, Ala. A specimen was obtained 14 hours post mortem, 3 weeks after a third-trimester abortion. Fetal membranes from a normal pregnancy at term were obtained fresh and snap-frozen in isopentane and liquid nitrogen. Sections of 4 μm were cut at −20° C, fixed in acetone for 15 minutes, and then stored at −70° C until processed.

After thawing for 1 hour at room temperature, sections were processed in a similar manner as described for paraffin-embedded tissues, starting with permeabilization.

Colostrum and breast milk. Breast milk was collected from a total of 25 healthy postpartum women according to a protocol approved by the Human Research Advisory Committee. Group I (n = 8) consisted of first milk samples only; colostrum, collected at the time of delivery or up to 3 days post partum. Group II (n = 7) consisted of samples collected 3 to 7 days post partum. Group III (n = 10) samples were collected 5 to 25 weeks post partum. The milk was centrifuged at 2000 rpm for 20 minutes and fat was removed prior to storage (−70° C). CA 125 levels were determined with standard test kits (supplied by Centocor, Malvern, Pa.). Protein concentrations were determined according to the method of Bradford[20] with reagents (from Biorad Inc., Richmond, Calif.).

Results

In the embryo (approximately 6 weeks' gestation) CA 125 antigen was detected by antibodies M 2 and M 11 in the periderm and the amnion (Fig. 1). In formalin-fixed sections CA 125 antigen was not detected by the OC 125 antibody. Nanbu et al.[17] previously described CA 125 in these tissues in a 7-week-old embryo with the use of OC 125 antibody and frozen sections. Inasmuch as formalin-fixation per se may be destructive or cause masking of some epitopes, we compared staining in frozen and formalin-fixed sections of term amnionic membranes. In frozen sections, OC 125, M 2, and M 11 all stained the amnion. However, formalin fixation and paraffin embedding of this tissue resulted in a marked decrease in the intensity of staining by OC 125, whereas staining intensity produced by antibodies M 2 and M 11 was much less affected (Fig. 1).

In sections of formalin-fixed embryonic tissue, CA 125 antigen is distinctly localized intracellularly in the notochord, enteric duct remnants (vitelline and allantoic ducts), umbilical cord, myocardium, and pericardium by M 2 and M 11 antibodies (Fig. 2). Extracellular localization of the antigen was detected surrounding the mesonephric duct (wolffian duct), the upper gastrointestinal tract, and possibly on the surface of the trophoblast by M 2 and M 11 antibodies (Fig. 2). OC 125 poorly stained or did not stain these tissues.

In the adult, CA 125 antigen was detected in peridermal derivatives: the apocrine sweat glands and the mammary glands. In formalin-fixed sections of apocrine sweat glands from the axilla, CA 125 antigen is detected intracellularly by antibodies M 2 and M 11, but not by OC 125 (Fig. 3). In frozen sections of lactating mammary glands (provided by the Cooperative Human Tissue Network), antigen was detected by OC 125 and M 2 antibodies intraductally (Fig. 4). Considering the intraductal location of antigen in lactating mammary glands, as opposed to its intracellular localization in the apocrine sweat glands, CA 125 might be anticipated to be present in breast milk. On assay, very high levels of antigen were found in colostrum (Group I, n = 8), ranging from 15,000 to 60,000 U/ml with a mean of 34,213 ± 5904 U/ml. CA 125 antigen levels

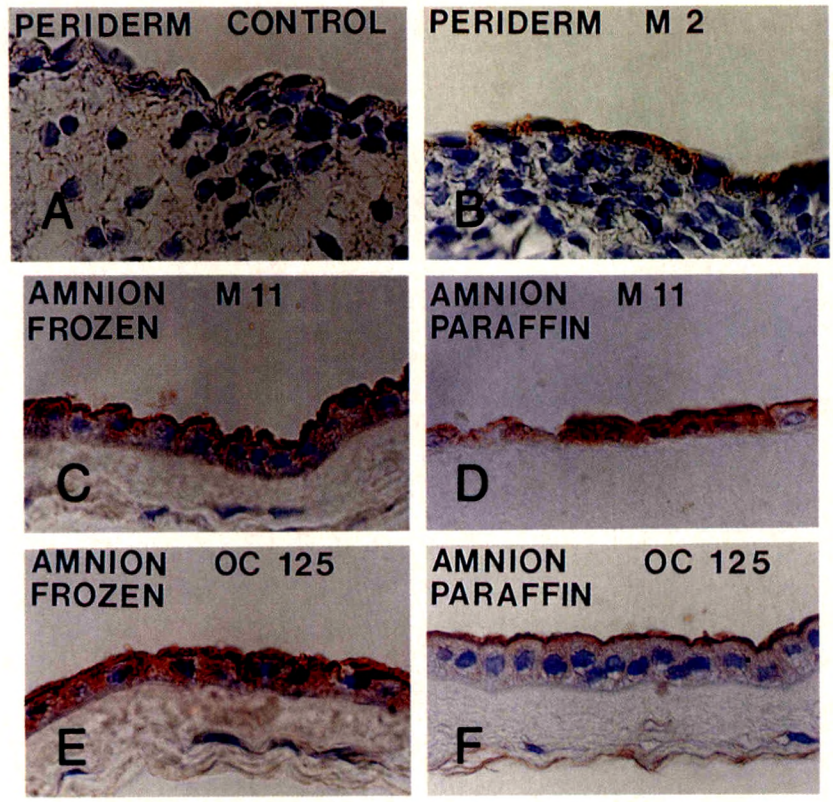


Fig. 1. Fetal membranes from normal pregnancy at term. **A**, Periderm; mouse ascites control. **B**, Periderm; M 2 antibody. CA 125 antigen is localized primarily intracellularly throughout epithelial surface of embryo; periderm, as recognized by antibody M 2. **C**, Fetal membranes frozen and acetone-fixed; M 11 antibody. Staining is more intensely associated with cell surface. (Original magnification ×1000.) **D**, Fetal membranes formalin-fixed and paraffin-embedded; M 11 antibody. Maximal intensity of staining is seen on cell surfaces along with some less prominent intracellular staining. (Original magnification ×1000.) **E**, Fetal membranes frozen and acetone-fixed specimen; OC 125 antibody. Intense staining of cell surfaces along with some intracellular staining is observed. (Original magnification ×1000.) **F**, Fetal membranes formalin-fixed and paraffin-embedded; OC 125 antibody. There is slight, but clear, staining of cell surfaces. (Original magnification ×1000.)

in groups II and III were much lower, ranging from 1130 to 1755 U/ml (mean, 1469 ± 92 U/ml) and 28 to 203 U/ml (mean, 105 ± 16 U/ml), respectively (Fig. 5). Statistically significant differences in CA 125 antigen levels between groups I and II and between groups II and III ($p < 0.001$) were determined with the Student t test (Fig. 5). Mean protein levels in groups I, II, and III were 45, 12, and 9 mg/ml, respectively. CA 125 specific activity (units CA 125 per milligram protein) ranged from 478 to 1395 in group I, 82 to 160 in group II, and 3 to 27 in group III (Fig. 5).

Comment

With the use of the newly developed antibodies M 2 and M 11, we confirm Nanbu's observation of CA 125 antigen in embryonic periderm and amnion. Colocalization of these new monoclonal antibodies with OC 125 in various tissues, support the notion that the new antibodies recognize epitopes that are closely associated with those recognized by OC 125. Whereas the antigen recognized by OC 125 has not been completely described, it is generally considered to be a high molecular weight glycoprotein (>200,000 daltons).[20] The configuration of the immunoradiometric assay used to measure CA 125 (Centocor, Malvern, Pa.) with single monoclonal antibody (OC 125) in a sandwich assay, implies that the CA 125 molecule contains more than one epitope for antibody binding. Independent data using immunoprecipitation and western blotting indicate that the epitopes recognized by OC 125, M 2, and M 11 reside on a common molecular species of approximately 250,000 daltons (unpublished data).

Because antibodies M 2 and M 11, but not always OC 125, detected antigen in the embryonic periderm and

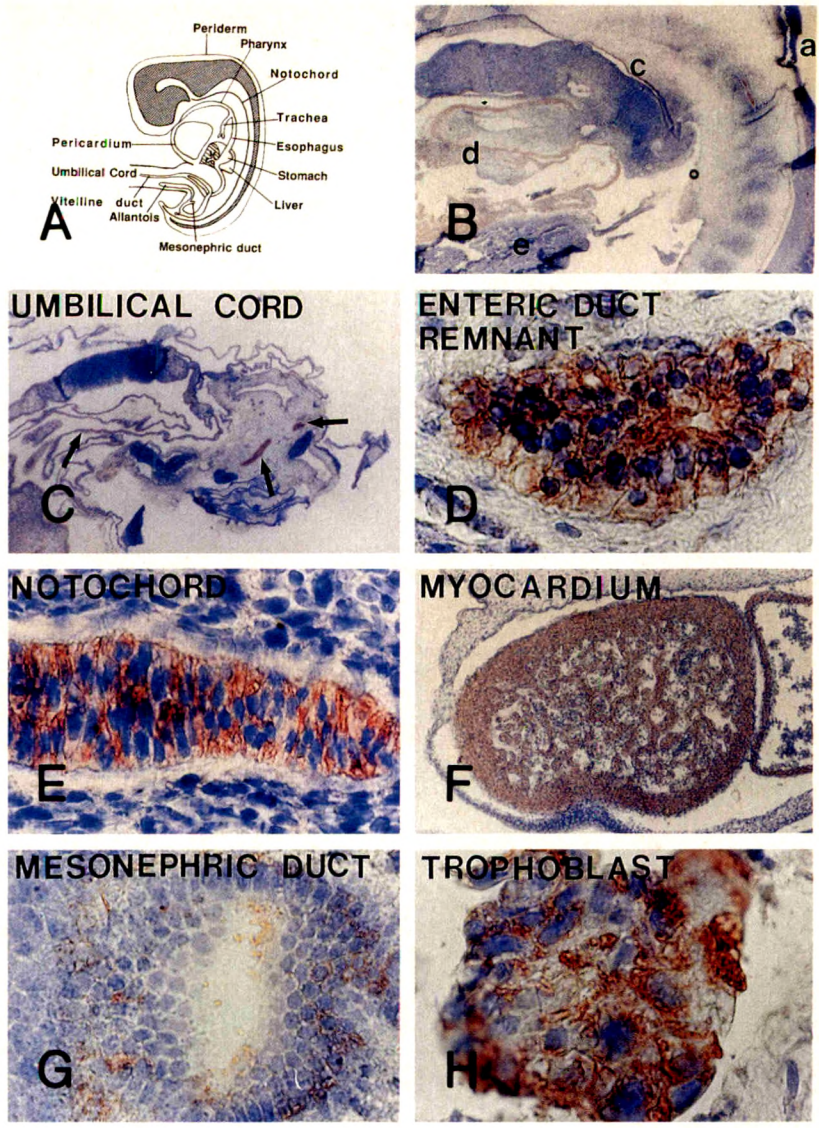


Fig. 2. Embryo, gestational age approximately 6 weeks, formalin-fixed and paraffin embedded. **A,** Human embryo, approximately 6 weeks old in hemisection, illustrating periderm, notochord, endodermal canal, pericardium, and liver. **B**, Human embryo, approximately 6 weeks old. **a**, Neural tube; **b**, notochord; **c**, pharynx; **d**, myocardium; **e**, liver. **C**, Umbilical cord; with enteric duct remnants (vitelline duct and allantoic duct) both showing immunoreactivity intracellularly when exposed to M 2 and M 11 antibodies. Also illustrated is amnion with prominent staining. (Original magnification ×100.) **D**, Enteric duct remnant in umbilical cord at increased magnification, illustrating clearly intracellular distribution of antigen. (Original magnification ×1000.) **E**, Notochord, cut tangentially, reacts with both M 2 and M 11 antibodies. Staining produced is evenly distributed intracellularly. (Original magnification ×1000.) **F**, Myocardium, ventriculus to left and atrium to right. Staining is produced by M 2 and M 11 antibodies inside myocardial cells and in pericardium *(bottom right)*. (Original magnification ×400.) **G**, Mesonephric duct. Epitopes recognized by M2 and M 11 antibodies are localized on cell surfaces, encircling this structure, along with some less prominent localization intraluminally. (Original magnification ×1000.) **H**, Fragment of trophoblastic tissue shows reactivity to both M2 and M 11 antibodies. Apparently staining is localized intracellularly and extracellularly. (Original magnification ×1000.)

amnion in formalin-fixed and paraffin-embedded sections, we speculate that epitopes recognized by OC 125 were either masked or damaged by the formalin-fixative or damaged by the hot paraffin (60° C) in routinely processed specimens. Accordingly, we divided the term amnion in two parts and processed one as a frozen specimen and the other routinely in formalin and paraffin. Although OC 125 antibody does detect the antigen in formalin-fixed term amnion in which the antigen is abundantly present, it is strikingly less prominent than when the tissue is frozen. The intensity of staining obtained by M 2 and M 11 was much less af-

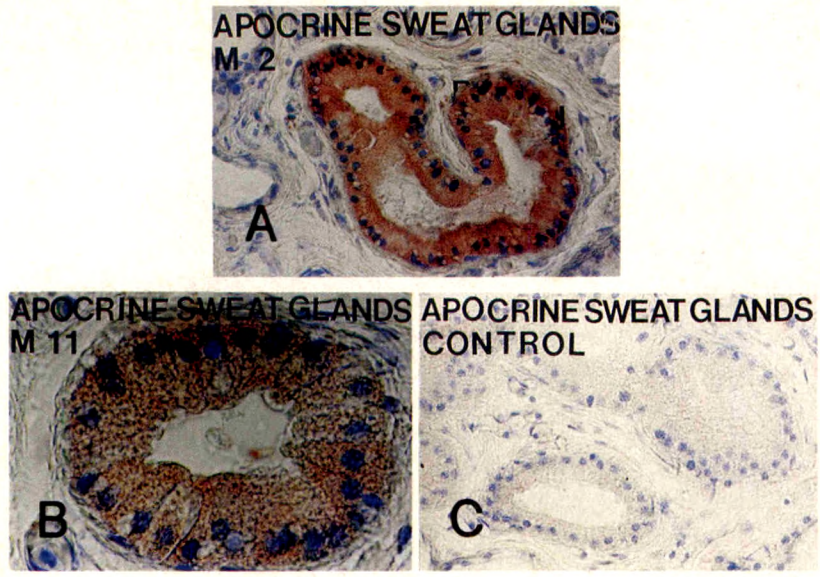


Fig. 3. Normal adult apocrine sweat glands from axilla, formalin-fixed and paraffin-embedded tissue sections. **A**, CA 125 antigen is detected intracellularly in secretory epithelium of apocrine sweat glands by M 2 antibody. (Original magnification ×400.) **B**, Apocrine sweat glands at higher magnification show same pattern produced by M 11 antibody. (Original magnification ×1000.) **C**, Apocrine sweat glands; mouse ascites control. (Original magnification ×400.)

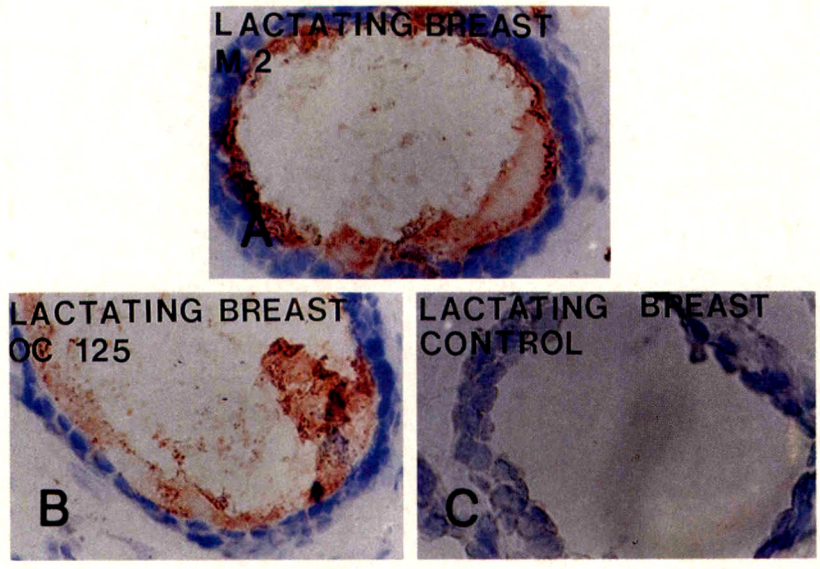


Fig. 4. Normal lactating mammary glands, frozen and acetone-fixed tissue sections. (Original magnification ×1000.) **A**, Lactating mammary glands; M 2 antibody. In lactating breast CA 125 antigen is detected in secretory product intraductally by M 2 antibody, but not intracellularly in epithelial cells. **B**, Lactating mammary glands; OC 125. OC 125 antibody produces similar staining pattern as M 2 antibody. **C**, Lactating mammary glands; mouse ascites control.

fected by formalin fixation and paraffin embedding. We conclude that the epitopes recognized by M 2 and M 11 are less subject to loss when the tissue is formalin fixed and paraffin embedded, compared with the epitopes recognized by OC 125. When CA 125 antigen is found in abundance in tissues the method of processing is not critical; however, when antigen is present in relatively low abundance, as may be the case in early embryonic development, processing of the specimen may be of significant importance.

Our results show a widespread distribution of CA 125 antigen in tissues that arise from all three embryonic germ layers. CA 125 was detected from the ectoderm in the periderm, mammary glands, and apocrine sweat glands; CA 125 was detected from the mesoderm in the myocardium and the mesonephric duct; and CA

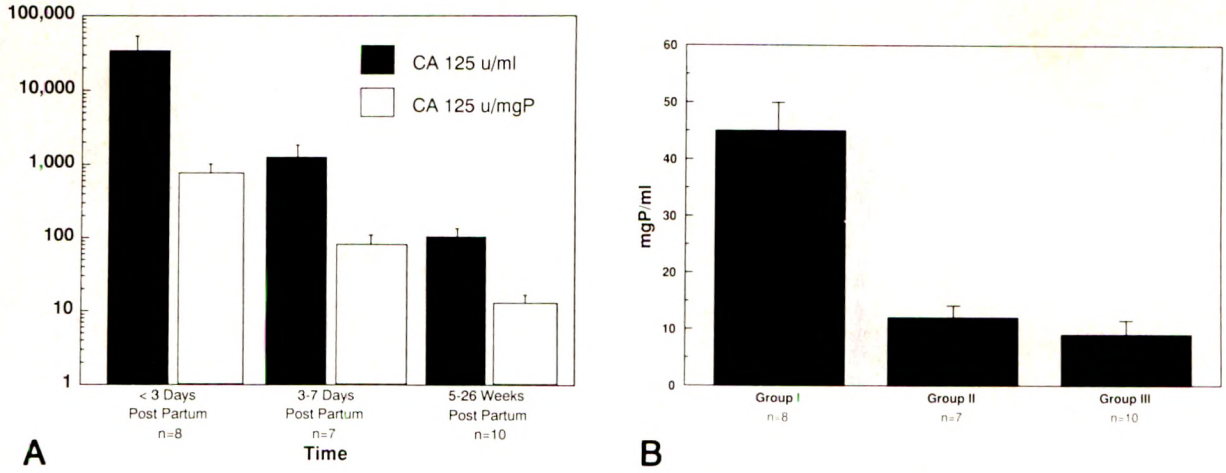


Fig. 5. CA 125 and protein levels in colostrum and breast milk. **A**, CA 125 levels and specific activity (units per milligram protein) in colostrum and breast milk at 0 to 3 days, 3 to 7 days and 5 to 26 weeks after parturition. **B**, Protein levels in colostrum and breast milk.

125 was detected from the endoderm in the enteric ducts (vitelline and allantoic ducts) and the gastrointestinal tract. Such a wide distribution of the antigen in developing tissues indicates an important role for CA 125 during early embryonic development and may ultimately help us to understand why it is produced by various malignancies later in life.

The observation of CA 125 on embryonic surfaces (i.e., the periderm, the amnion, the enteric duct remnants, the gastrointestinal tract, and the mesonephric duct) led us to speculate a potential role for this molecule in the prevention of cell-to-cell adhesion (i.e., prevention of collapse or closure of these structures). In the adult, the antigen is found on the surface of cells that line the pericardium, pleura, and peritoneum suggesting that CA 125 antigen may serve the same role in these organs during adult life.

Hanisch et al.[21] described CA 125 in a single sample of breast milk collected 4 days post partum. In this study, CA 125 was measured in colostrum and milk at various stages of lactation. High concentrations of CA 125 were found in colostrum and early milk, declining rapidly during the first week of lactation and reaching a steady level in mature milk. Colostrum is known to have some antiinfective properties and protect the offspring from various infections, especially gastrointestinal infections. This property is attributed mainly to the presence of secretory immunoglobulin A, which is the main protein in colostrum,[22] but also to other substances such as lysozyme, lactoferrin, and lactoperoxidase. Secretory immunoglobulin A is found in high concentrations in early milk, as described by McClelland et al.[23] and then falls rapidly to a low but constant level in a fashion similar to CA 125. Whether this association has a clinical significance or whether CA 125 contributes to the antiinfective properties of colostrum is unknown.

The presence of CA 125 antigen in apocrine glands raises the question of its role in the secretory process per se. Glands with an apocrine mode of secretion, which are known to express CA 125 antigen, include endometrial glands,[16] bronchoalveolar glands,[19] mammary glands, and apocrine sweat glands. Other glands (i.e., eccrine sweat glands), which are the same embryonic origin as apocrine sweat glands (ectodermal), do not express CA 125 antigen. This could imply a different function of these sweat glands or be related to different secretory processes.

The expression of CA 125 antigen in the early developing embryo and in the secretory products of apocrine glands suggests a broad developmental or biologic function that remains to be elucidated. Cloning of the CA 125 gene and structural analysis of the gene product may provide further insight into the biology of this antigen.

We wish to acknowledge the generous contributions of OC 125 antibody and CA 125 IRMA kits by Vincent Zurowski, Jr., of Centocor, and the contribution of tissue specimens from the Cooperative Human Tissue Network, Southwestern Division, Katherine Sexton, Tissue Procurement, University of Alabama at Birmingham.

REFERENCES

1. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest 1981;68:1331.
2. Kabawat SE, Bast RC Jr, Welch WR, Knapp RC, Calvin RB. Immunopathologic characterization of a monoclonal antibody that recognizes common surface antigens of hu-

man ovarian tumors of serous, endometroid, and clear cell types. Am J Clin Pathol 1983;79:98-104.
3. Mainguene C, Aillet G, Kremer M, Chatal JF. Immunohistochemical study of ovarian tumors using the OC 125 monoclonal antibody as a basis for potential in vivo and in vitro applications. J Nucl Med Allied Sci 1986;30:19-22.
4. Koelma IA, Nap M, Rodenburg CJ, Fleuren GJ. The value of tumour marker CA 125 in surgical pathology. Histopathology 1987;11:287-94.
5. Bast RC Jr, Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983;309:883-7.
6. Kuzuya K, Nozaki M, Chihara T. Evaluation of CA 125 as a circulating tumor marker for ovarian cancer. Acta Obstet Gynaecol Jpn 1986;38(6):949-57.
7. Vergote IB, Bormer OP, Abeler VM. Evaluation of serum CA 125 levels in the monitoring of ovarian cancer. Am J Obstet Gynecol 1987;157:88-92.
8. Patsner B, Mann WJ, Cohen H, Loesch M. Predictive value of preoperative serum CA 125 levels in clinically localized and advanced endometrial carcinoma. Am J Obstet Gynecol 1988;158:399-402.
9. Lindgren J, Kuusela P, Hellstrom PE, Petterson T, Klockars M. The ovarian cancer associated antigen CA 125 in patients with pleural effusions. Eur J Cancer Clin Oncol 1988;24(4):737-9.
10. Halila H, Stenman UH, Seppala M. Ovarian cancer antigen CA 125 levels in pelvic inflammatory disease and pregnancy. Cancer 1986;57:1327-9.
11. Barbieri RL, Niloff JM, Bast RC Jr, Schaetzl E, Kistner RW, Knapp RC. Elevated serum concentrations of CA 125 in patients with advanced endometriosis. Fertil Steril 1986;45(5):630-4.
12. Barbieri RL. CA 125 and endometriosis. Contrib Gynecol Obstet 1987;16:103-8.
13. Takahashi K, Yamane Y, Kijima S, Yoshino K, Shibukawa T, Kitao M. CA 125 antigen is an effective diagnostic for external endometriosis. Gynecol Obstet Invest 1987;23:257-60.
14. Barbati A, Anceschi MM, Alberti P, Pomili G, Renzo GC, Cosmi EV. Ontogeny of CA 125 antigen in pregnancy: immunoradiometric determination in amniotic fluid and immunohistochemical localization in fetal membranes. Am J Obstet Gynecol 1989;160:514-7.
15. O'Brien TJ, Hardin JW, Bannon GA, Norris JS, Quirk JG Jr. CA 125 antigen in human amniotic fluid and fetal membranes. Am J Obstet Gynecol 1986;155:50-5.
16. Quirk JG Jr, Brunson GL, Long CA, Bannon GA, Sanders MM, O'Brien TJ. CA 125 in tissues and amniotic fluid during pregnancy. Am J Obstet Gynecol 1988;159:644-9.
17. Nanbu Y, Fujii S, Konishi L, Nonogaki H, Mori T. CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion. Am J Obstet Gynecol 1989;161:462-7.
18. Kabawat SE, Bast RC Jr, Bhan AK, Welch WR, Knapp RC, Colvin RB. Tissue distribution of a coelomic-epithelium-related antigen recognized by the monoclonal antibody OC 125. Int J Gynecol Pathol 1983;2:275-85.
19. Nouwen RL, Pollet DE, Eerdekens MW, Hendrix PG, Briers TW, DeBroe ME. Immunohistochemical localization of placental alkaline phosphatase, carcinoembryonic antigen and cancer antigen CA 125 in normal and neoplastic human lung. Cancer Res 1986;46:866-76.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein dye binding. Anal Biochem 1976;72:248-54.
21. Davis HM, Zurowski VR Jr, Bast RC Jr, Klug TL. Characterization of the CA 125 antigen associated with human epithelial ovarian carcinomas. Cancer Res 1986;46:6143-8.
22. Hanisch FG, Uhlenbruck G, Dienst C, Stottrop M, Hippauf E. CA 125 and CA 19-9: two cancer-associated sialylsaccharide antigens on a mucus glycoprotein from human milk. Eur J Biochem 1985;149:323-30.
23. McClelland DBL, McGrath J, Sampson RR. Antimicrobial factors in human milk. Acta Paediatr Scand 1978;271 (suppl):1-20.

Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells

Jay H. Levin, MD, Sharon A. Tonetta, PhD, and Rogerio A. Lobo, MD
Los Angeles, California

Progestins stimulate prolactin production from endometrial stromal cells in culture. We compared the potencies of the synthetic progestins norethindrone and medroxyprogesterone acetate to natural progesterone in inducing stromal prolactin production. Modifications of the culture system provided an increase in stromal cell yield, thus permitting multiple comparisons from the treatment of cells from a common endometrial sample. The effects of high-dose estradiol also were evaluated in this system. The findings suggest relative potencies of 50:1 for medroxyprogesterone acetate and progesterone. Norethindrone gave intermediate and more variable responses. Estradiol potentiated prolactin production from only submaximal progestins doses. The differences between the progestin effects, in large measure, were due to differential culture growth as reflected by culture mass. Compared to controls and estradiol alone, all the progestins induced much greater prolactin production. Thus during decidualization, progestins probably promote both stromal growth and intracellular prolactin production. High-dose estradiol may not interfere with these events. (Am J Obstet Gynecol 1990;163:1932-8.)

Key words: Endometrium, stromal cells, prolactin, progestins

Human endometrium is known to undergo dramatic morphologic and functional changes after ovulation.[1, 2] In vitro models have shown that prolactin (PRL) is produced by decidualized endometrium,[3] increases with progestin stimulation,[4-6] and originates primarily from the stroma.[6-9] While it has been suggested that the synthetic progestins norethindrone and medroxyprogesterone acetate may be more potent than natural progesterone in stimulating stromal cells,[10] the relative potencies of these progestins for stimulating PRL production have not been demonstrated.

Estradiol has been shown to diminish the progestin-dependent stimulation of PRL production from follicular phase endometrial explants[5] as well as from stromal cell cultures when a high dose of estrogen was used.[11] In more recent studies using stromal cells, high-dose estrogen, such as achieved during controlled ovarian hyperstimulation, has been shown to enhance both medroxyprogesterone acetate[6] and progesterone[8] stimulatory effects on PRL production.

This study was designed to compare the potencies of norethindrone, medroxyprogesterone acetate, and progesterone for inducing endometrial decidualization as reflected by in vitro stromal PRL production, and to determine whether this secretion is due to increased cellular production or culture growth. Further, we wished to evaluate the effects a high dose of estradiol in this model. To this end, we modified existing culture systems[11-13] to increase cell yield to enable such parallel comparisons.

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Southern California School of Medicine.
Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, March 21-24, 1990, St. Louis, Missouri.
Reprint requests: Jay H. Levin, MD, Department of Obstetrics and Gynecology, Women's Hospital, Room 1M2, LAC/USC Medical Center, 1240 North Mission Road, Los Angeles, CA 90033.
6/6/24777

Material and methods

Four sets of cultures were performed from each tissue specimen obtained during the midluteal phase of two premenopausal, eumenorrheic women, ages 43 and 44. The endometrium was obtained immediately after hysterectomy for symptomatic uterine descensus. The midluteal phase status was documented by menstrual timing and confirmed histologically by the method of Noyes et al.[14]

Stromal cells were isolated from glands by a method adapted from that described by previous investigators.[11-13] The endometrial tissue was transported to the culture laboratory in refrigerated, sterile Dulbecco's modified Eagle's media (DMEM) (Gibco Laboratories, Grand Island, N.Y.) and minced within 30 minutes of removal from the uterus. Enzymatic digestion was then performed in a solution containing 0.1% collagenase, type II (Sigma Chemical Co., St. Louis), 0.1% hyaluronidase, type III (Sigma), 0.1% protease, type XIV (Sigma), and 0.1% ethylenedinaminetetraacetic acid

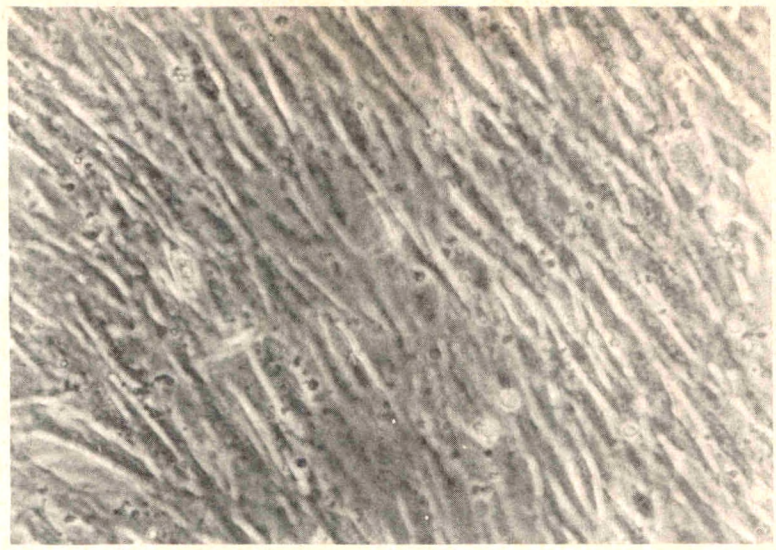


Fig. 1. Phase-contrast photomicrograph of stromal cells in culture.

(Sigma) for 30 minutes at 37° C with gentle stirring. After washing to quench the digestion process, the tissue was filtered sequentially through 75 μm and 38 μm sieves to obtain a highly enriched stromal cell suspension. This procedure yields 40 to 90 × 10^6 viable endometrial cells per specimen, with 80% to 90% total viability as determined by 0.4% trypan blue exclusion (Sigma).

Primary cultures were established with 10^6 viable cells per milliliter per culture well in DMEM with 10% fetal calf serum (Irvine Scientific, Santa Ana, Calif.), 5 μg/ml transferrin (Sigma), 10 μg/ml insulin (Sigma), penicillin, streptomycin, and fungizone (Gibco) at 37° C in a 95% air/5% carbon dioxide atmosphere. This concentration of cells resulted in efficient plating of purified stromal cell populations reaching confluence by 48 to 72 hours of culture. Purity of the cell population was confirmed by well-established morphologic criteria[15-17] (Fig. 1).

Media were changed every 48 hours for a total of 14 days. Steroid treatments were begun with initial plating and selected from the following: progesterone at doses of 50 and 500 ng/ml (P50 and P500); medroxyprogesterone acetate at doses of 10 and 100 ng/ml (M10 and M100); norethindrone at doses of 1, 10, and 100 ng/ml (N1, N10, and N100); estradiol at 5 ng/ml (E); and each of the progestin doses in combination with estradiol 5 ng/ml (P50E, P500E, M10E, M100E, N1E, N10E, and N100E). Controls consisted of media alone (C). In each experiment, controls and all treatment perturbations were performed in quadruplicate cultures. At the conclusion of the experiment, the cells were removed from the culture plates with a 0.1% solution of trypsin-ethylenediaminetetraacetic acid.

Collected media were assayed for PRL by a modification of the technique of Sinha et al.[18] The assay was performed with a 24-hour preincubation period and dilution of the anti-PRL antibody (AFP-2284C2 obtained from the National Institutes of Health, Bethesda, Md.) to the concentration of 1:200,000. Maximum binding ranged from 21% to 25%. This allowed an assay sensitivity of 400 pg/ml. Intraassay coefficients of variation were 15.4%, 7.3%, and 10.3% for the low, mid, and high ranges, respectively. The interassay coefficients of variation were 9.7%, 9.2%, and 9.9% for the low, mid, and high ranges, respectively. Mean PRL concentrations of pools at the low, mid, and high ranges were 1.2, 7.8, and 29 μg/ml, respectively. Samples containing levels of PRL greater than the assay's upper limit were diluted. Assay blanks generated counts consistent with <0.2 ng/ml.

Protein content was determined for each of the cultures. After trypsinization, the cultured cells were washed twice and stored at −70° C as pellets covered with buffered solution. The samples were then thawed and sonicated just before protein determination using the bicinchonincic acid protein assay[19] (Pierce Chemical Company, Rockford, Ill.).

Statistical testing included application of analysis of variance to test for differences between treatments and linear regression analysis was used to determine significant relationships.

Results

Prolactin production data (mean ± SD) for each of the quadruplicate culture experiments are depicted in Tables I and II and are henceforth referred to as experiments 1 and 2. Values for the initial 48 hours were all <400 pg/ml and are not depicted. Fig. 2 depicts the dynamics of this system. Stimulation with the highest

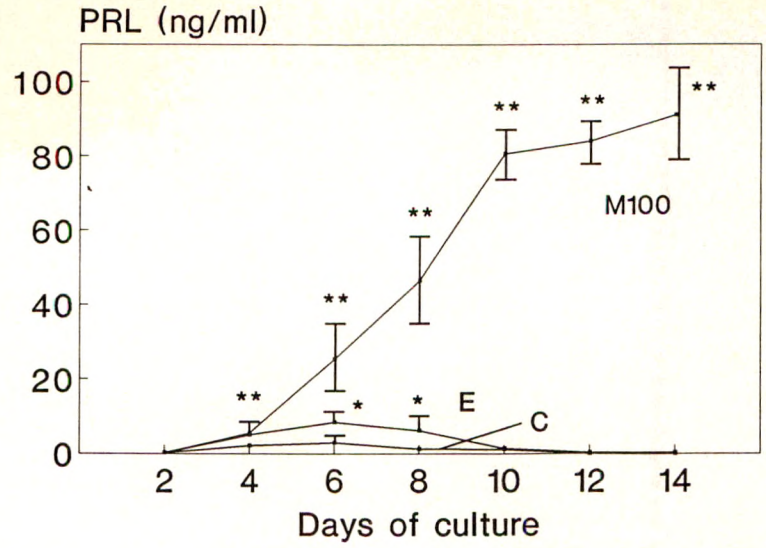


Fig. 2. PRL production curves for cells maximally stimulated with M100 compared with controls *(C)* and treatment with estradiol *(E)* 5 ng/ml. (By analysis of variance: *asterisk*, $p < 0.05$ vs *C*; *double asterisks*, $p < 0.05$ vs *E*.)

Table I. PRL production throughout experiment 1

	Day 4	*Day 6*	*Day 8**	*Day 10†*	*Day 12‡*	*Day 14‡*
Control	2.6 ± 0.6	3.9 ± 0.7	1.2 ± 0.3	<0.4	<0.4	<0.4
P50	2.5 ± 0.5	5.1 ± 1	6 ± 2	13 ± 2	23 ± 5	32 ± 9
M10	3.1 ± 0.8	9.8 ± 4	15 ± 6	60 ± 4	103 ± 4	119 ± 6
M100	3.5 ± 0.6	11.5 ± 4	17 ± 6	77 ± 3	137 ± 8	173 ± 14
P50E	2.4 ± 0.6	9.6 ± 2	19 ± 6	83 ± 5	160 ± 5	166 ± 18
M10E	2.9 ± 0.5	10.5 ± 3	17 ± 5	78 ± 2	171 ± 2	187 ± 25
M100E	3.6 ± 0.5	12.2 ± 3	21 ± 6	88 ± 4	172 ± 4	199 ± 14

Values expressed as mean ± SD.
*$p < 0.05$, all progestin treatments > control.
†$p < 0.05$, M100 and M10 > P50; and P50E > P50.
‡$p < 0.05$, M100 > M10 > P50; M10E > M10; and P50E > P50.

dose of medroxyprogesterone acetate (M100) resulted in exponentially increased PRL production, starting from day 4 to 6. Controls and estradiol alone resulted in only transient increases that peaked on day 6 and declined thereafter. The levels achieved in the controls and with estradiol alone were significantly lower than with any of the progestins.

Experiment 1. Experiment 1 compared P50, M10, M100, P50E, M10E, and M100E with controls (Table I). Prolactin production from control cultures peaked on day 6 at 3.9 ± 0.7 ng/ml and thereafter declined to <0.4 ng/ml by day 10. The progestins P50, M10, and M100 exerted continued stimulation to much higher levels (32 ± 9, 119 ± 6, and 173 ± 14 ng/ml) by day 14 (Fig. 3). Addition of estradiol to each of these progestins resulted in day 14 levels of 166 ± 18, 187 ± 25, and 199 ± 14 ng/ml for P50E, M10E, and M100E, respectively (Fig. 3). Thus M100 stimulation was greater than M10 by 45% ($p < 0.05$), and both M100 and M10 were more potent than P50 by 440% ($p < 0.05$) and 272% ($p < 0.05$), respectively. In addition, although estradiol potentiated both P50 (by 419%; $p < 0.05$) and M10 (by 57%; $p < 0.05$), M100 remained unaffected (p = NS). These dose relationships and the potentiation by estradiol both became apparent by day 6 and were statistically significant at day 10 (Table I).

Protein content of these cultures was 360 ± 25, 328 ± 44, 367 ± 32, 513 ± 20, 456 ± 16, 522 ± 79, and 558 ± 54 μg per culture for control, P50, M10, M100, P50E, M10E, and M100E, respectively (Table III). Dividing PRL production by protein content accounted for some but not all the differences in PRL production. For M100 this ratio was greater than for both controls ($p < 0.05$) and P50 ($p < 0.05$) but not for M10 (p = NS) (Table III). Therefore when compared with controls and P50, M100 stimulated both greater culture mass and greater PRL production per mass.

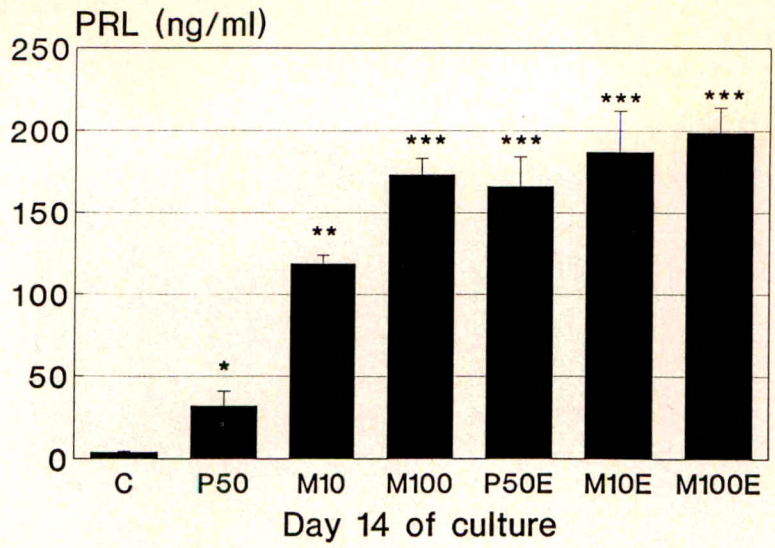


Fig. 3. Comparison of day-14 PRL production from cultures in experiment 1. (By analysis of variance: *asterisk*, $p < 0.05$ vs controls *[C]; double asterisks*, $p < 0.05$ vs P50 and *C; triple asterisks*, $p < 0.05$ vs M10, P50, and *C;* all steroid stimulations significantly greater than *C.*)

Table II. PRL production throughout experiment 2

	Day 4	*Day 6**	*Day 8**	*Day 10†*	*Day 12†*	*Day 14‡*
Control	2.1 ± 1.2	2.6 ± 1.1	1.2 ± 0.2	1.1 ± 1.0	<0.4	<0.4
P50	3.4 ± 0.5	12 ± 2	26 ± 6	60 ± 7	68 ± 7	58 ± 3
P500	3.5 ± 0.4	16 ± 4	35 ± 9	69 ± 3	82 ± 8	72 ± 10
M10	4.6 ± 0.5	20 ± 4	39 ± 9	66 ± 4	76 ± 7	83 ± 13
M100	5.4 ± 0.8	25 ± 9	47 ± 13	81 ± 8	84 ± 5	91 ± 14
N1	2.9 ± 0.5	9 ± 3	23 ± 9	38 ± 12	50 ± 6	54 ± 9
N10	3.3 ± 0.4	13 ± 3	31 ± 8	56 ± 8	65 ± 5	76 ± 9
N100	3.4 ± 0.3	16 ± 5	34 ± 8	68 ± 5	64 ± 2	81 ± 6
E	5.0 ± 0.5	8.4 ± 0.8	6.1 ± 3.3	1.4 ± 0.2	<0.4	<0.4
P50E	3.5 ± 0.2	15 ± 3	32 ± 8	63 ± 13	71 ± 4	64 ± 12
P500E	1.0 ± 0.3	4 ± 1	13 ± 2	38 ± 8	49 ± 7	45 ± 7
M10E	2.5 ± 0.9	10 ± 6	29 ± 12	56 ± 9	73 ± 15	70 ± 13
M100E	4.3 ± 0.6	21 ± 5	43 ± 15	70 ± 7	84 ± 11	88 ± 17
N1E	3.3 ± 0.5	16 ± 5	38 ± 12	63 ± 6	69 ± 2	77 ± 14
N10E	3.6 ± 0.1	18 ± 2	42 ± 13	68 ± 4	71 ± 5	79 ± 10
N100E	3.3 ± 0.6	20 ± 9	33 ± 14	48 ± 5	54 ± 10	61 ± 8

Values are expressed as mean ± SD.
*$p < 0.05$, E > control.
†$p < 0.05$, N1 < N10; N1E > N1.
‡$p < 0.05$, N1 < N10; P50 < P500; and N1E > N1.

When compared with M10, however, M100 stimulated only greater increases in culture mass with equivalent relative PRL production.

For control cultures this ratio was very much lower than for any of the progestin treatments, suggesting a failure to induce comparable PRL production, even when compared to P50, which resulted in similar final culture mass. However, there was a good correlation between protein content and day-14 PRL production ($r = 0.79$; $p < 0.001$).

Experiment 2. Experiment 2 compared P50, P500, M10, M100, N1, N10, and N100 alone and with estradiol to control and estrogen (Table II). Production from controls peaked by day 6 at 2.9 ± 1.1 ng/ml and thereafter declined <0.4 ng/ml by day 10. The progestins P50, P500, M10, M100, N1, N10, and N100 stimulated much greater production of 58 ± 3, 72 ± 10, 83 ± 13, 91 ± 14, 54 ± 9, 76 ± 9, and 81 ± 6 ng/ml by day 14, respectively (Fig. 4). Addition of estradiol to each of these progestins resulted in day-14 levels of 64 ± 12, 45 ± 7, 70 ± 13, 88 ± 17, 77 ± 15, 79 ± 10, and 61 ± 8 ng/ml, respectively. Estradiol alone provided only transient stimulation that peaked at 8.4 ± 0.7 ng/ml by day 6. This was greater than for controls

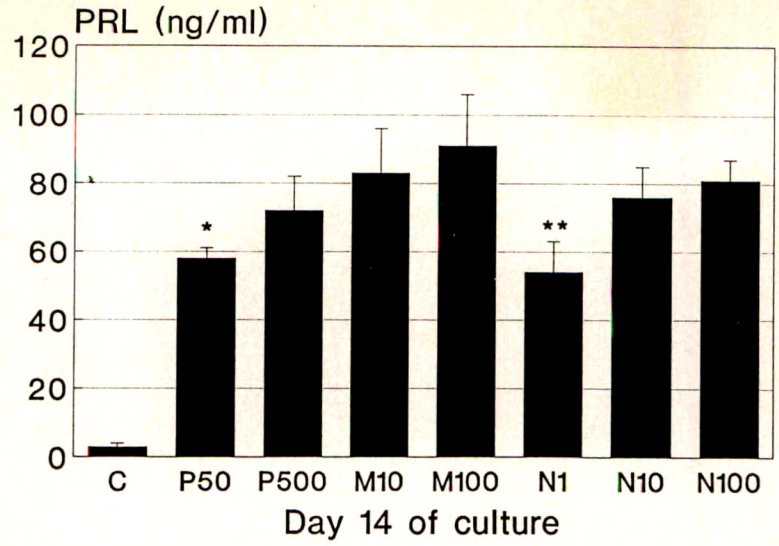


Fig. 4. Comparison of day-14 PRL production from cultures in experiment 2. (By analysis of variance: *asterisk*, $p < 0.05$ vs P500, M10, M100, N10, and N100; *double asterisks*, $p < 0.05$ vs N10, N100, M10, M100, and P500; all progestin stimulations greater than *C*.)

Table III. Protein content and day-14 PRL production/protein ratios for experiment 1

	*Protein** (μg/culture)	*PRL/protein ratio†* (ng/μg)
Control	360 ± 25	<0.001
P50	328 ± 44	0.102 ± 0.033
M10	367 ± 32	0.330 ± 0.0[illegible]
M100	513 ± 20	0.349 ± 0.025
P50E	456 ± 16	0.345 ± 0.036
M10E	522 ± 79	0.355 ± 0.089
M100E	558 ± 54	0.351 ± 0.051

Values are expressed as mean ± SD.
*$p < 0.05$, M100 > M10; M10E > M10; and P50E > P50.
†$p < 0.05$, M10 = M100; P50 < M10.

Table IV. Protein content and day-14 PRL production/protein ratio for experiment 2

	*Protein** (μg/culture)	*PRL/protein ratio†* (ng/μg)
Control	87 ± 9	<0.004
P50	130 ± 12	0.449 ± 0.045
P500	159 ± 9	0.454 ± 0.071
M10	159 ± 25	0.535 ± 0.127
M100	163 ± 7	0.564 ± 0.120
N1	102 ± 11	0.514 ± 0.070
N10	128 ± 11	0.601 ± 0.093
N100	148 ± 22	0.568 ± 0.073
E	116 ± 9	<0.004
P50E	178 ± 23	0.374 ± 0.109
P500E	148 ± 30	0.286 ± 0.029
M10E	173 ± 15	0.400 ± 0.050
M100E	177 ± 12	0.503 ± 0.117
N1E	165 ± 12	0.464 ± 0.084
N10E	178 ± 10	0.447 ± 0.065
N100E	150 ± 9	0.406 ± 0.033

Values are expressed as mean ± SD.
*$p < 0.05$, control, E, and N1 < all other treatments; N1E > N1.
†$p < 0.05$, control and E < < other treatments.

($p < 0.05$) but much less than for any of the progestins.

As depicted in Fig. 4, N1 was less potent than both N10 (by 29%, $p < 0.05$) and N100 (by 33%; $p < 0.05$), but N10 was equipotent to N100. P50, which was equal to N1, exerted less stimulation than M10 (29%; $p < 0.05$) and M100 (36%; $p < 0.05$) as well as N10 and N100. Although not rigorously significant by analysis of variance, isolated *t* tests revealed that P500 was more potent than P50 (72 ± 10 vs 58 ± 3; $p < 0.05$). Additionally, P500 stimulation equaled that of M10, N10, and N100.

When added to the progestins, estradiol significantly potentiated only N1 (77 ± 15 vs 54 ± 9; $p < 0.05$). Estradiol had no such dramatic effect on the other progestin doses. Although M10E and N100E had slightly lower production than M10 and N100, these differences were not significant (p = NS). P500E exerted less stimulation than P500 ($p < 0.05$), but appeared to be an aberrant, isolated observation. The other progestins of equal or greater potency were not affected negatively. Prolactin production for P500E already had fallen by day 4, which was before any differential stimulation should have been manifest in this model (Table II).

The protein content of these cultures are listed in Table IV. For the progestin treatments, differences in PRL production were paralleled by the differences in culture mass. The addition of estradiol potentiated

growth only for the least potent progestin doses, P50 ($p < 0.05$), N1 ($p < 0.05$), and N10 ($p < 0.05$). The protein content correlated well with day-14 PRL production ($r < 0.58$; $p < 0.001$), and further normalizing PRL production to culture mass diminished the apparent differences in progestin responses. However, differential culture mass again could not explain the very large differences in PRL production between the progestins and controls or estrogen.

Comment

Endometrial stromal PRL production, which serves as a marker of decidualization, responds to progestin stimulation in a dose-dependent manner.[8] Tseng et al.[10] reported relative potencies of 135:90:70 for medroxyprogesterone acetate:norethindrone:progesterone in the stimulation of aromatase activity from luteal stromal cells cultured for 3 days. Other comparisons of progestin potencies rely upon results obtained from cultures of different tissue specimens. We wished to assess the progestin potencies for stimulating production of PRL in a system designed to compare differential responses of cells obtained from a common tissue.

This study confirmed the dose-response nature of the progestin stimulation of stromal cells and has established relative potencies for norethindrone and medroxyprogesterone acetate compared with progesterone in stimulating stromal PRL production. Our findings suggest that norethindrone and medroxyprogesterone acetate are equipotent at a dose of 10 ng/ml, but that medroxyprogesterone acetate (and not norethindrone) exerts a greater stimulation at a higher dose (100 ng/ml). Both of the synthetic progestins at 10 ng/ml resulted in stimulation statistically indistinguishable from natural progresterone at 500 ng/ml. Thus the relative potency ratio for medroxyprogesterone acetate:progesterone is suggested to be 50:1, with more variable potency responses occurring with norethindrone (5- to 50-fold greater) compared to progesterone.

Although estrogens may increase endometrial progesterone receptors in vitro[20] and in vivo,[21] the role of luteal estrogens in the regulation of stromal prolactin production remains less clear. Previous work has suggested that depending on the dose used in culture, estrogens alone may stimulate,[8, 11] have no effect,[6, 11] or inhibit slightly[11] this stromal function. When combined with progestins, estradiol has been shown to exert negative,[5] neutral,[6] or positive[8, 9] effects.

This study has shown that estradiol at 5 ng/ml may stimulate low levels of stromal PRL production, but that this effect is transient. Furthermore, while estradiol may potentiate the progestins, this effect was limited to the lower progestin doses. While both P50 and N1 stimulation may be enhanced by estradiol, M100 was not. Results from the progestin doses of intermediate potency (P500, M10, N10, and N100) were more variable. In one case M10 was potentiated by estradiol, whereas in other cultures none of the intermediate progestins were enhanced. This suggests that high levels of luteal estrogens may influence endometrial decidualization but that this may occur only during submaximal progestin stimulation.

In this model plating equal numbers of cells to each culture served to normalize the system. Therefore the relative protein content reflected differences in culture "growth," which may have been from cell proliferation, cell enlargement, or deposition of extracellular matrix. That the "growth" trends paralleled PRL production differences suggests that for the different progestin stimulations much of the differential secretory activity was linked to the differences in culture mass. However, in control and estrogen only cultures, the ratios of peak PRL production/protein content were far less than for any of the progestin treatments. Thus, the dramatic stimulation by progestins compared to control and estrogen most probably resulted from induction of cellular PRL production capability and not from the relatively smaller differences in "growth."

In conclusion, we have established relative potencies for progestin stimulation of endometrial stromal PRL production. This appears to be dependent on both culture "growth" and induction of PRL synthesis. Estradiol alone has little effect, but may actually enhance both of these aspects of culture dynamics under conditions of submaximal progestin stimulation.

REFERENCES

1. Healy DL, Hodgen GD. The endocrinology of human endometrium. Obstet Gynecol Surv 1983;38:509-30.
2. Maslar IA. The progestational endometrium. Semin Reprod Endocrinol 1988;2:115-28.
3. Maslar IA, Riddick DH. Prolactin production by human endometrium during the normal menstrual cycle. Am J Obstet Gynecol 1979;135:751-4.
4. Maslar IA, Ansbacher R. Effects of progesterone on decidual prolactin by organ cultures of human endometrium. Endocrinology 1986;118:2102-8.
5. Daly DC, Maslar IA, Riddick DH. Prolactin production during in vitro decidualization of proliferative endometrium. Am J Obstet Gynecol 1983;145:672-8.
6. Huang JR, Tseng L, Bischof P, Janne OA. Regulation of prolactin production by progestin, estrogen, and relaxin in human endometrial stromal cells. Endocrinology 1987; 121:2011-7.
7. Braverman MB, Bagni A, de Ziegler D, Den T, Gurpide E. Isolation of prolactin-producing cells from first and second trimester decidua. J Clin Endocrinol Metab 1984; 58:521-5.
8. Irwin JC, Kirk D, King RJB, Quigley MM, Gwatkin RB. Hormonal regulation of human endometrial stromal cells in culture: an in vitro model for decidualization. Fertil Steril 1989;52:761-8.
9. Randolph JF, Peegel H, Ansbacher R, Menon KMJ. In vitro induction of prolactin production and aromatase activity by gonadal steroids exclusively in the stroma of separated proliferative human endometrium. Am J Obstet Gynecol 1990;162:1109-14.
10. Tseng L, Mazella J, Sun B. Modulation of aromatase activity in human endometrial stromal cells by steroids, tamoxifen, and RU486. Endocrinology 1986;118:1312-8.
11. Pavlik EJ, Katzenellenbogen BS. Human endometrial cells

in primary tissue culture: estrogen interactions and modulation of cell proliferation. J Clin Endocrinol Metab 1978;47:333-44.
12. Fleming H, Gurpide E. Growth characteristics of primary cultures of stromal cells from human endometrium. J Steroid Biochem 1982;16:717-20.
13. Liu H-C, Tseng L. Estradiol metabolism in isolated human endometrial epithelial glands and stromal cells. Endocrinology 1979;104:1674-81.
14. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3-25.
15. Centola GM, Cisar M, Knab DR. Establishment and morphologic characteristics of normal human endometrium in vitro. In Vitro 1984;20:451-62.
16. Mandelande T, Rochefort H. A 51K progestin-regulated protein secreted by human endometrial cells in primary culture. J Clin Endocrinol Metab 1987;64:1294-1301.
17. Osteen KG, Hill GA, Hargrove JT, Gorstein F. Development of a method to isolate and culture highly purified populations of stromal and epithelial cells from human endometrial biopsy specimens. Fertil Steril 1989;52:965-72.
18. Sinha YN, Selby FW, Lewis UJ, Van der Loan WP. Homologous radioimmunoassay for human prolactin. J Clin Endocrinol Metab 1973;36:509-16.
19. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76-85.
20. Eckert RL, Katzenellenbogen BS. Human endometrial cells in primary tissue culture: modulation of the progesterone receptor level by natural and synthetic estrogens in vitro. J Clin Endocrinol Metab 1981;52:699-708.
21. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarthy KS Jr. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab 1988;67:334-46.

A rabbit model for bacteria-induced preterm pregnancy loss

Raymond A. Dombroski, MD,[a] Donna S. Woodard, PhD,[b] Michael J. K. Harper, PhD,[a] and Ronald S. Gibbs, MD[a]

San Antonio, Texas

Bacterial infection has been implicated in premature labor in humans. To elucidate mechanisms and potential intervention strategies, we sought to develop a model of infection-induced pregnancy loss in rabbits. On day 21 (70% of gestation), each uterine horn was inoculated hysteroscopically with 0.2 ml containing saline solution or 10^6 cfu *Escherichia coli* or *Bacteroides bivius* or *Fusobacterium necrophorum*. Fetal viability was assessed. Animals were sacrificed at various times or as delivery occurred. Serum progesterone and amniotic fluid prostaglandins were measured. Cultures and histologic sections were prepared. Compared with the saline solution group, *E. coli* and *F. necrophorum*–inoculated rabits were significantly more likely to deliver (16 of 16 and six of seven with mean times of 31.9 ± 10.7 and 28.3 ± 11.5 hours, respectively for *E. coli* and *F. necrophorum*). Positive amniotic fluid cultures for the *E. coli* group were found in 11 of 12 (92%) and for the *F. necrophorum* group in three of three cases (100%). Histologic inflammation was seen heavily in both the *E. coli* and *F. necrophorum* groups, whereas it was absent in the saline solution group. Inoculation with *B. bivius* led to a much lower pregnancy loss rate (eight of 32) and less histologic inflammation despite positive uterine cultures in most animals. This model may provide an opportunity to determine mechanisms of clinical or subclinical intraamniotic infection and to test intervention strategies. (Am J Obstet Gynecol 1990;163:1938-43.)

Key words: Premature labor, infection, rabbit, hysteroscopy, histology

Considerable data suggest that bacterial infection may be a cause of preterm birth. Histologic chorioamnionitis and positive membrane cultures are more common after preterm birth,[1] and various bacteria and their products have been found in the amniotic fluid of women with preterm labor.[2, 3] Bacterial lipopolysaccharides, moreover, are capable of stimulating the production of prostaglandins, platelet-activating factors, and cytokines in intrauterine and other tissues.[4, 5]

Systemic administration, including intravenous and intraperitoneal routes, of bacterial endotoxin to pregnant animals has been shown to precipitate pregnancy loss in several species.[6-9] In a rabbit model we tested the hypothesis that intrauterine bacterial infection causes preterm pregnancy loss. The methods reported represent a novel approach to the study of infection-induced preterm birth.

From the Department of Obstetrics and Gynecology[a] and Pathology,[b] The University of Texas Health Science Center at San Antonio.
Supported by a grant from the Texas Higher Education Committee.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, March 15-18, 1989, San Diego, California.
Reprints not available.
6/6/24751

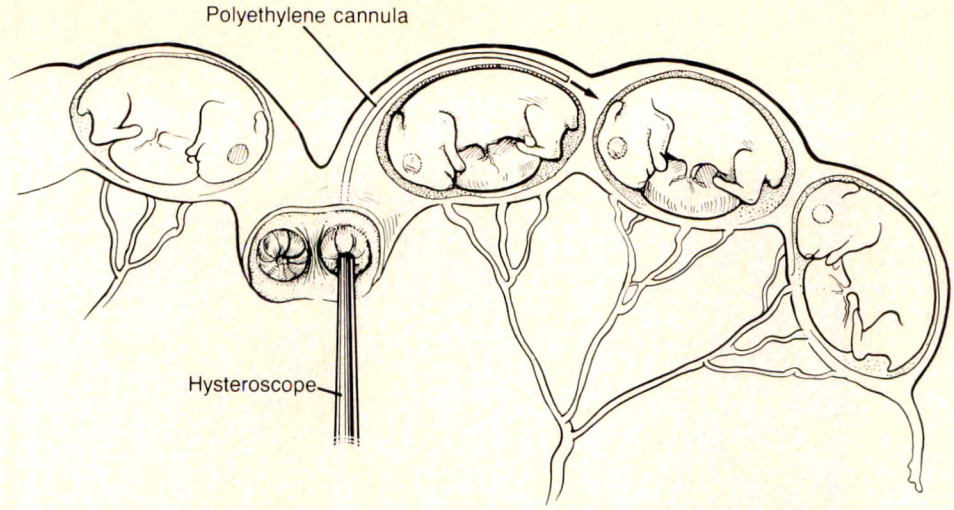


Fig. 1. Schematic representation of experimental inoculation of uterine horns.

Material and methods

Virgin Cambridge-New Zealand White Cross rabbits (weight >3 kg) were obtained from a local vendor (Penn Acres Ranch, Wimberly, Tex.). The animals were housed in the animal facility with a 12-hour-day/12-hour-night cycle and provided water as desired and feed (170 gm of rabbit pellets per day). After acclimatization for a minimum of 2 weeks, artificial insemination, followed immediately by an intravenous injection of 50 IU human chorionic gonadotropin, was performed. At this facility, these methods result in a pregnancy rate of 95%.

On day 21 of gestation (normal gestational length, 33 to 34 days), does were anesthetized with an intramuscular injection of a mixture of ketamine-promethazine-xylazine (0.5 ml/kg). After vaginal preparation with a povidone-iodine solution, a Storz 27020B hysteroscope (provided by Storz Instrument, St. Louis) was introduced into the vagina and the cervices were visualized. Through the operating port, a 0.06-inch outside diameter polyethylene cannula was introduced transcervically into each horn of the uterine cavity a minimum of 6 cm (Fig. 1). The same injection of either a bacterial inoculum (see below) or pyrogen-free saline solution (0.2 ml) was then made into each horn.

Bacterial inocula were prepared for the following: *Escherichia coli* ATCC 12014, *Bacteroides bivius* ATCC 29303, and *Fusobacterium necrophorum* ATCC 25286. For preparation of the *E. coli* inoculum, a tube of thioglycollate medium was inoculated aerobically and grown for 6 to 8 hours before concentration and washing. *B. bivius* and *F. necrophorum* were grown in chopped meat broth for 12 to 16 hours. These latter cultures were maintained and washed under strict anaerobic conditions. For each organism, media were removed by centrifugation and the bacterial pellet was washed (by resuspension and recentrifugation) three times in phosphate-buffered saline solution before final suspension in an appropriate volume of buffer to yield 10^7 to 10^8 cfu/ml. Because the volume of the inoculum was 0.2 ml, each horn was injected with approximately 10^6 to 10^7 cfu. Final suspensions were plated semiquantitatively before inoculation in each case to confirm colony count. Finally, a remaining aliquot was plated to check for viability after the 1- to 2-hour interval required for inoculation.

In a pilot experiment with *E. coli* to evaluate inoculum size, no difference was observed between inocula of 10^3 to 10^4 and 10^7 to 10^8 cfu per horn.

After inoculation animals were observed for signs of sepsis and delivery. Animals were killed by an intravenous injection of 5 ml pentobarbital sodium when delivery was recognized or after 7 days. Rectal temperatures were taken at the time of operation, 24 hours later, and when put to death.

When animals were put to death, blood, peritoneal fluid, chorioallantoic-amniotic fluid (when available), and decidua were cultured aerobically and anaerobically on 5% sheep blood agar, chopped meat broth, and thioglycollate broth. Gram stains of amniotic fluid, peritoneal fluid, and decidua were prepared. The viability of fetuses remaining in utero was noted. A blood sample was obtained for progesterone determination, which was performed in a routine radioimmunoassay.[10] Amniotic fluid was obtained for measurements of prostaglandins F_2 and E_2. The prostaglandins were measured by the radioimmunoassay procedure described by Harper et al.[11] and modified by Jones et al.[12] without prior extraction. The antibodies were obtained from Advanced Magnetics Inc. (Cambridge, Mass.). The in-

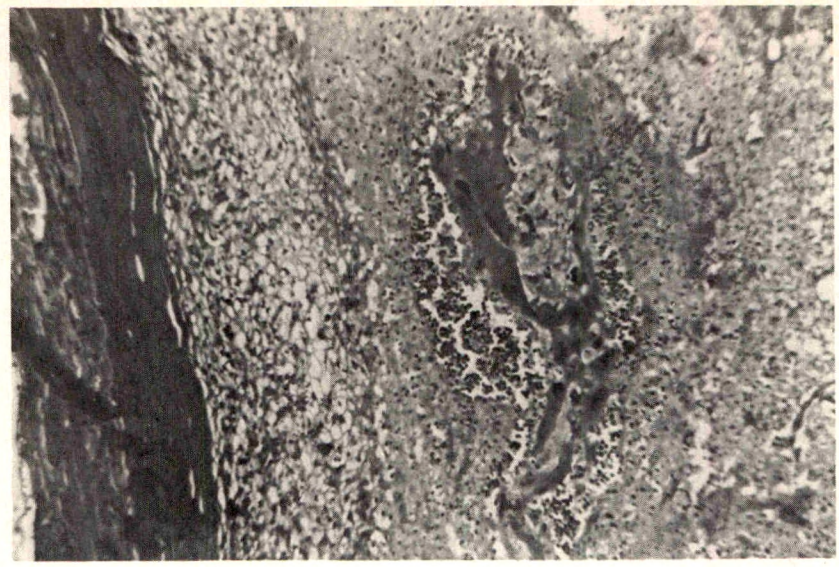


Fig. 2. Representative histologic section of placental zone of separation from *E. coli*—inoculated animal when put to death shows heavy infiltrate of polymorphonuclear leukocytes. (Hematoxylin and eosin. Original magnification ×40.)

Table I. Pregnancy outcomes by intrauterine inoculum

Inoculum	*n*	*Delivered (No.)*	*Died (No.)*	*Time to delivery (hr, mean ± SD)*	*No. with live fetuses*	*Purulent exudate in utero (No.)*
Saline solution	9	2	0	134 ± 19.8	7	2
E. coli	16	16*	0	31.9 ± 10.7†	0	7
F. necrophorum	7	6‡	3	28.3 ± 11.5†	0	1
B. bivius	32	8	0	90.5 ± 39.5	28	10

*$p < 0.0001$, compared with number delivered in saline solution group.

†$p < 0.05$ for *E. coli* and *F. necrophorum* groups compared with saline solution group for mean time to delivery, by Newman-Keuls method.

‡$p < 0.05$, compared with number delivered in saline solution group.

terassay and intraassay coefficients of variations were, 7% and 8.7%, respectively, for prostaglandin F and 11.5% and 8.8% for prostaglandin E. Indomethacin (10 μg/ml) was added to sample tubes before collection. Prostaglandins were not extracted before assay because pilot work demonstrated no difference between values with and without extraction. A portion of the uterine horn containing a placenta was fixed in 10% neutral buffered formalin for histologic examination.

Histologic descriptions used standard terms.[13, 14] Categoric data were analyzed by uncorrected χ^2 or Fisher's exact test. Interval and ratio measurements were compared by one-way analysis of variance and Newman-Keuls multiple comparisons or Student's *t* statistic when appropriate. All tests were two-tailed and for independent samples. When assumptions were not met, equivalent nonparametric statistics (Kruskall-Wallis analysis of variance, Mann-Whitney U) were used. Statistical analyses were performed on an IBM PC with the True Epistat program.

Results

When put to death, five animals (7.2%) were found to have had inadvertent perforation of the uterus and were excluded from further analysis (saline solution group, one animal; *B. bivius*, three, *E. coli*, none, *F. necrophorum*, one). Pregnancy outcomes are shown in Table I. Additional pertinent observations were noted. In the two animals delivered in the saline solution group, both also had several dead fetuses remaining in utero. Both also contained bloody amniotic fluid and grossly purulent uterine contents. In the remaining seven rabbits, all undelivered fetuses were alive; there was no purulence.

In the *F. necrophorum* group, abruptio placentae was noted in three animals with dead fetuses remaining in utero. Uterine horns and placentas appeared pale in all seven rabbits in this group.

In the *B. bivius* group, eight animals had delivery of one or more pups (Table I). Of these, six had one or more live pups remaining in utero when put to death, but two of the six also contained dead fetuses.

Table II. Development of fever* by intrauterine inoculum

Group	*No. of animals with fever*		*p (vs saline solution)*	
	At 24 hr	*When put to death*	*At 24 hr*	*When put to death*
Saline solution	0 (n = 9)	0 (n = 9)		
E. coli	6 (n = 12)	6 (n = 10)	0.02	0.01
F. necrophorum	2 (n = 6)	2 (n = 4)	0.14	0.08
B. bivius	1 (n = 32)	5 (n = 32)	0.78	0.27

Fever was defined as rectal temperature ≥104.00° F; see text. Numbers in parentheses represent total number of animals at this time.

Table III. Culture results by intrauterine inoculum

Group	*Culture site*								
	Amniotic fluid			*Peritoneal fluid*			*Decidua*		
		Positive			*Positive*			*Positive*	
	n	*No.*	*%*	*n*	*No.*	*%*	*n*	*No.*	*%*
Saline solution	9	1	11	9	1	11	9	2	22
E. coli	12	11	92	12	9	75	Not done		
F. necrophorum	3	3	100	4	0	0	4	4	100
B. bivius	30	10	33	32	0	0	29	23	79

No. positive is the number of animals that were culture positive for the organisms inoculated. In the saline solution group one grew enterococci at all three sites and one grew enterococci in decidua only. Both of these animals delivered offspring.

When overall mean temperatures at 24 hours and when put to death were compared with those at time of operation, we found a small but significant increase at these times only in the saline solution group (102.01° ± 0.39° F at operation vs 102.42° ± 0.31° at 24 hours vs 102.61° ± 0.43° F when put to death, $p < 0.05$), but not in the other groups. Although there was no significant temperature change overall in the *B. bivius* group, the mean temperatures were higher when put to death in the eight animals that delivered than in the 24 that did not (104.16° ± 1.77° vs 102.70° ± 0.71°, respectively; $p = 0.02$).

Because of wide variance in the three bacterial groups, we analyzed temperature changes alternatively. If fever in rabbits was defined as 104.00° F (i.e., mean of all temperatures at operation + 2 SD), analysis by inoculum is shown in Table II.

Culture results are shown in Table III. Rabbits inoculated with *E. coli* and *F. necrophorum*, if positive, cultured only the organism inoculated. In the *B. bivius* group, several animals had positive cultures for other organisms. Three cultured enterococci as the only organism at any site in decidua. These do not appear in Table III. None of these animals delivered any offspring. Three other animals had additional organisms isolated together with *B. bivius* at the same site or a different one. One of these grew enterococci from chorioallantoic-amniotic fluid, peritoneal fluid, and decidua alone with *B. bivius* in the decidua. This animal delivered offspring. A second animal had both *B. bivius* and enterococci in the decidua. In the third, γ-streptococci were isolated from amniotic fluid and decidua in combination with *B. bivius*. Neither of these two animals delivered offspring.

Table IV. Serum progesterone level when animals were put to death, classified by intrauterine inoculum

Group	*n*	*Serum progesterone (ng/ml, mean ± SD)*
Saline solution	7	11.20 ± 9.19*
E. coli	7	5.79 ± 5.42*
F. necrophorum	4	5.99 ± 1.20*
B. bivius	25	7.91 ± 3.62*
Delivered	6	5.81 ± 0.89†
Undelivered	19	8.57 ± 3.91†

*Differences between these four groups are not significant by Kruskall-Wallis analysis.

†The difference between these two subgroups is ($p = 0.03$) by Mann-Whitney U test but may reflect a difference in gestational age.

Progesterone levels were obtained from several animals when put to death (Table IV). Kruskall-Wallis analysis showed no differences between groups (saline solution, *E. coli*, *F. necrophorum*, or *B. bivius*), although variances within groups were considerable. In a separate analysis by Mann-Whitney U test, the difference between the subgroups of *B. bivius* animals (i.e., deliv-

Table V. Chorioallantoic-amniotic fluid prostaglandin E_2 and $F_{2\alpha}$ levels

Group	*n*	*Prostaglandin E_2**	*Prostaglandin $F_{2\alpha}$**
Saline solution	6	786.5 ± 878	351.3 ± 322
E. coli	9	1951.4 ± 796	2802.8 ± 2120
B. bivius	13	915.5 ± 549	755.2 ± 770
Delivered	4	1103.2 ± 543	1201.7 ± 1316
Undelivered	9	832.0 ± 562	556.7 ± 312

*Values for *E. coli* group are significantly higher as compared with the saline solution and *B. bivius* groups ($p < 0.05$) by Newman-Keuls multiple comparisons. Differences between delivered and undelivered subgroups of *B. bivius*–inoculated rabbits are not significantly different by Mann-Whitney U test.

ered vs undelivered) reached statistical significance ($p = 0.03$) but may reflect a difference in gestational age.

Chorioallantoic-amniotic fluid prostaglandin levels when put to death are shown in Table V. The levels of both prostaglandins were significantly elevated in *E. coli*–treated animals compared with all other groups. The differences observed between delivered and undelivered *B. bivius* animals were not significant. Again, variances are great and sample sizes are small.

The histologic findings varied considerably by inoculating organism. Saline solution–treated animals that remained undelivered showed no inflammatory exudate in any layers. Occasionally, margination of white blood cells was noted in subplacental vessels. *E. coli*–treated animals displayed heavy infiltration of the endometrium, amanion, and chorion with polymorphonuclear leukocytes (Fig. 2). Scatterd foci of polymorphonuclear leukocytes were found in the zone of separation, decidua, and subplacenta. Scattered inflammatory cells were found within maternal sinuses of the placenta but the overall vascular anatomy of the placenta was maintained.

Animals inoculated with *F. necrophorum* displayed inflammation and necrosis of the endometrial glandular elements. Infiltration by white blood cells of the membranes was prominent, but the zone of separation and the subplacenta were generally spared. The most striking findings consisted of extensive abruptio placenta and engorgement of maternal vascular channels with the placenta.

B. bivius–treated animals showed inflammatory cells in all layers, although the degree of inflammation was less than with other inocula. The placenta was generally spared.

Comment

In these experiments we developed an animal model for bacterial-induced pregnancy loss. We chose hysteroscopic introduction, rather than transabdominal inoculation, to mimic ascending infection more closely. We chose *E. coli* because of its clinical importance in maternal and neonatal infection and because it produces large amounts of endotoxin (lipopolysaccharide). Subsequently, we chose *B. bivius* and *F. necrophorum* because of their frequent isolation either in clinically evident intraamniotic infection in preterm pregnancies[15] or from amniotic fluid in patients in preterm labor.[2]

We have demonstrated that hysteroscopic introduction of *E. coli* and *F. necrophorum* into the rabbit uterine cavity at approximately 70% of gestation regularly led to pregnancy loss, with a mean of 30 hours. In saline solution–inoculated animals, bacteria, presumably from the lower genital tract, were occasionally introduced into the uterine cavity, which was accompanied by pregnancy loss. However, in the absence of such inadvertent introduction, atraumatic, hysteroscopic introduction of saline solution did not result in pregnancy loss.

With *E. coli* and *F. necrophorum* inoculation, pregnancy loss was accompanied by fever in the does, microbiologic and histologic confirmation of infection-inflammation when animals were put to death, fetal death, and occasionally, maternal death. Because there was considerable variance in progesterone levels when animals were put to death, we observed no significant differences in mean levels by inoculation group.

However, because serum progesterone levels may truly be lower in infected animals, it is tempting to speculate. Perhaps a rise in serum prostaglandin $F_{2\alpha}$ (or at least in uterine vein levels) could adversely affect luteal function and thus decrease progesterone levels. In the rabbit, luteolysis probably occurs systematically via prostaglandin $F_{2\alpha}$ in the venous drainage from the uterus and not, for example, via lymphatic drainage to the renal or iliac lymph nodes.[16, 17]

Mean amniotic fluid prostaglandin levels were elevated in all groups inoculated with bacteria compared with the saline solution group, but because of small numbers and wide variance, these increases achieved significance only for the *E. coli* group.

A different response was observed in animals inoculated with *B. bivius*, compared with the *E. coli* and *F. necrophorum* groups. Although the organism was detectable in the decidua in most animals (79%), it was less commonly isolated from the chorioallantoic-amniotic fluid (33%) and was accompanied by less his-

tologic inflammation. Compared with *E. coli* and another gram-negative anaerobe *(F. necrophorum)*, *B. bivius* led to a milder clinical response. Overall, the clinical response in the *B. bivius* group was not dissimilar from the saline solution group. However, if the *B. bivius* group is compared with the noncontaminated saline solution controls, then *B. bivius* inoculation presents a clinical picture reminiscent of subclinical infection—positive cultures, low-grade inflammation, mild maternal symptoms, occasional pregnancy loss, and absence of fetal death in utero.

Depending on the organisms selected, this model may provide an opportunity to explore the mechanism of clinical intraamniotic infection or infection-induced premature birth. The model may be further used to test intervention strategies.

We thank Barry Korinick and Patricia St. Clair for their technical assistance.

REFERENCES

1. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1988;319:972-8.
2. Romero R, Sirtori M, Oyarzun E, et al. Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. Am J Obstet Gynecol 1989;161:817-24.
3. Romero R, Roslansky P, Oyarzun E, et al. Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. Am J Obstet Gynecol 1988;158:1044-9.
4. Romero R, Hobbins JC, Mitchell MD. Endotoxin stimulates prostaglandin E_2 production by human amnion. Obstet Gynecol 1988;71:227-8.
5. Bennett PR, Rose MP, Myatt L, Elder MG. Preterm labor: stimulation of arachidonic acid metabolism in human amnion cells by bacterial products. Am J Obstet Gynecol 1987;156:649-55.
6. Zahl PA, Bjerknes C. Induction of decidua-placental hemorrhage in mice by the endotoxins of certain gram-negative bacteria. Proc Soc Exp Biol Med 1943;54:329-32.
7. Rioux-Darrieulat F, Parant M, Chedid L. Prevention of endotoxin-induced abortion by treatment of mice with antisera. J Infect Dis 1978;137:7-13.
8. Skarnes RC, Harper MJK. Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. Prostaglandins 1972;1:191-201.
9. Kullander S. Fever and parturition: an experimental study in rabbits. Acta Obstet Gynecol Scand 1977;66 (suppl):77-85.
10. Pauerstein CJ, Eddy CA, Croxatto HD, Siler-Khodr TM, Croxatta HB. Temporal relationships of estrogen, progesterone, and luteinizing hormone levels to ovulation in women and infrahuman primates. Am J Obstet Gynecol 1978;130:876-84.
11. Harper MJ, Norris CJ, Friedrichs WE, Moreno A. PolyI:C accelerates ovum transport in the rabbit by a prostaglandin-mediated mechanism. J Reprod Fertil 1981;63:81-9.
12. Jones MA, Cao Z, Anderson W, Norris C, Harper MJ. Capillary permeability changes in the uteri of recipient rabbits after transfer of blastocysts from indomethacin-treated donors. J Reprod Fertil 1986;78:261-73.
13. Davies J. Histochemistry of the rabbit placenta. J Anat 1956;90:135-42.
14. Mossman HW. The rabbit placenta and the problem of placental transmission. Am J Anat 1926;37:433-97.
15. Sperling RS, Newton ER, Gibbs RS. Intraamniotic infection in low-birth-weight infants. J Infect Dis 1988;157: 113-7.
16. Head JR, Lande IJM. Uterine lymphatics: passage of ink and lymphoid cells from the rat's uterine wall and lumen. Biol Reprod 1983;28:941-55.
17. Hunter GL, Casida LE. Absence of local effects of the rabbit uterus on weight of corpus luteum. J Reprod Fertil 1967;13:179-81.

In vitro inhibition of esterase activity in amniotic fluid: Comparison with bacterial cultures

Iffath Abbasi Hoskins, MD, Joseph Katz, PhD, Faith J. Frieden, MD, Steven A. Ordorica, MD, and Bruce K. Young, MD

New York, New York

Assessment of leukocyte esterase activity in amniotic fluid for the rapid and reliable diagnosis of chorioamnionitis has been demonstrated previously. We compared in vitro inhibition of esterase activity in amniotic fluid with bacterial cultures to identify the origins of the specific esterases released by the infecting organisms. One hundred forty-one samples were tested (90 uninfected, 51 infected). Each sample was evaluated for Gram stain, cultures, and an in vitro esterase assay followed by ebelactone inhibition. Forty-two patients had positive amniotic fluid cultures. Ebelactone produced varying degrees of inhibition of esterase activity (range, 20% to 60%) in the uninfected samples and in those infected with gram-negative organisms. There was no inhibition in the samples infected with gram-positive organisms. Thus different groups of bacteria may elicit the production of different and specific esterases in infected amniotic fluid, as shown by the differences in in vitro inhibition. (Am J Obstet Gynecol 1990;163: 1944-7.)

Key words: Chorioamnionitis, leukocyte esterase, inhibition, bacterial cultures

Chorioamnionitis is a common diagnostic dilemma for the obstetrician. Rapid and accurate diagnosis poses the most significant problem. The clinical diagnosis is usually based on a constellation of symptoms that include maternal fever, tachycardia, uterine tenderness, foul odor of amniotic fluid, leukocytosis, and fetal tachycardia. However, there is uncertainty with regard to accurate and early diagnosis and laboratory confirmation.[1]

Because leukocytes are known to be released in response to infections, examination of the amniotic fluid for their presence may be useful in the evaluation for chorioamnionitis. The neutrophilic leukocytes released contain several esterases that are not present in serum, urine, or body tissues.[2, 3]

We have previously described the reliability of an in vitro spectrophotometric assay of leukocyte esterase activity in amniotic fluid for the prediction of chorioamnionitis. In this study we compared in vitro inhibition of this activity with the results of bacterial cultures in an attempt to identify the origins of the specific esterases that are released by the infecting organisms.

Material and methods

Sterile specimens of amniotic fluid were obtained from 141 antepartum patients who had undergone transabdominal amniocenteses during the second and third trimesters for genetic studies, rhesus isoimmunization, and fetal lung maturity studies, or to rule out chorioamnionitis. All patients included in the study had intact membranes. Ninety samples were obtained from uninfected patients and 51 from women with chorioamnionitis as determined by the presence of at least two of the following criteria: maternal pyrexia (temperature >37.8° C), leukocytosis (white blood cell count >12,000/mm^3), uterine tenderness, and fetal tachycardia (heart rate >160 beats/min). Each sample was divided into three parts: one each for Gram stain, aerobic and anaerobic cultures, and the in vitro esterase activity. The in vitro assay was performed by adding known amounts of amniotic fluid (10 λ) to solutions containing 20λ of a 5 μmol/L solution of *p*-nitrophenyl hexanoate in dimethyl sulfoxide and 1 ml of buffer (0.1 mol/L phosphate, pH 7.4). The change in optical density at wavelength 405 nm ($\Delta OD_{405}/10^{-3}$) was calculated from the initial slopes of the change in absorbance at 405 nm as a function of time. One unit of activity was defined as a change of 1 absorbance unit/min. Then, 10 λ of a nonspecific esterase inhibitor ebelactone (1 mg/ml) was added to each aliquot, and the $OD_{405}/10^{-3}$ was again calculated to measure the degree of inhibition of esterase activity in the sample. The results were compared with those obtained from amniotic fluid aerobic and anaerobic cultures.

Gram staining was performed with commercial reagents (crystal violet, safranin, and Gram's iodine; Difco Laboratories, Detroit) under standard conditions. The presence of white blood cells and microorganisms was noted, and a semiquantitative assessment was made

From the Department of Obstetrics and Gynecology, New York University Medical Center.

Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.

Reprint requests: Iffath Abbasi Hoskins, MD, Department of Obstetrics and Gynecology, New York University Medical Center, 550 First Ave., Room 9N1, New York, NY 10016.

6/6/24775

Table I. Results of parameters used to detect chorioamnionitis

	Gram stain		*Cultures*		*Leukocyte esterase activity* (range)*	*Ebelactone* (range)*
	Pos.	*Neg.*	*Pos.*	*Neg.*		
Uninfected (n = 90)						
Second trimester (15-20 wk) (n = 58)	1	57	0	58	2.8-7.0†	0.9-3.7†
Third trimester (28-40 wk) (n = 32)	3	29	1	31	3.1-6.8†	1.3-3.9†
Infected (n = 51)						
Second trimester (15-20 wk) (n = 19)	7	12	13	6	17.0-18.3†	9.0-17.8†
Third trimester (28-38 wk) (n = 32)	18	14	28	4	17.3-19.6†	7.6-18.2†

*Absorbance units.
†$p < 0.05$.

Table II. Reliability of amniotic fluid diagnostic parameters

	Sensitivity (%)		*Specificity (%)*		*Positive predictive value (%)*		*Negative predictive value (%)*	
	Second trimester	*Third trimester*	*Second trimester*	*Third trimester*	*Second trimester*	*Third trimester*	*Second trimester*	*Third trimester*
Gram stain	36.8	56.3	98.3	90.6	87.5	85.7	82.6	67.4
Cultures	68.4	87.5	100	96.9	100	96.6	90.6	88.6

after examination of 20 high-power fields. A positive Gram stain was defined as one containing any microorganisms per high-power field.

The amniotic fluid aliquots for cultures were collected in plastic, capped syringes and transported to the laboratory immediately after collection for inoculation into the culture media. Positive cultures were defined as those containing one or more types of organisms in excess of 10^4 cfu/ml. For purposes of this study, if more than one organism was isolated on culture, the predominant organism was labeled the causative one. A positive leukocyte esterase enzyme assay was defined as a value >12 enzyme activity units.[4]

The sensitivity, specificity, and predictive values for the diagnostic parameters were calculated. A χ^2 analysis was performed to compare the results of the esterase assay and the inhibition assay between the uninfected and infected groups. Statistical significance was defined as a p value <0.05.

Results

The relationship between the Gram stain, cultures, leukocyte esterase activity, and inhibition in the uninfected and infected samples is shown in Table I. There were 90 uninfected amniotic fluid samples; 58 were obtained during the second trimester. One sample showed gram-positive cocci on Gram stain, there were no positive amniotic fluid cultures, and the leukocyte esterase activity of all samples was within the normal ranges as previously defined (>12 enzyme activity units). Three of the 32 uninfected samples obtained in the third trimester had positive Gram stains (two cases with gram-positive cocci and one with gram-negative rods). One of these samples also showed a positive amniotic fluid culture with organisms >10^4 cfu/ml (group B streptococcus).

Table III. Pathogens identified on amniotic fluid cultures

Organism	*Uninfected (n = 90)*	*Infected (n = 51)*
Aerobic gram-positive		
Group B streptococci	1	6
Enterococci	0	10
Anaerobic gram-positive		
Streptococci	0	0
Aerobic gram-negative		
E. coli	0	21
Enterobacteriaceae	0	0
Anaerobic gram-negative		
B. fragilis	0	3
F. nucleatum	0	1

Nineteen of the 51 infected samples were obtained from second-trimester amniotcenteses. Of these, only seven had positive Gram stains (gram-positive cocci, three cases; gram-negative rods, three; gram-negative cocci, one). All seven samples also had positive amniotic fluid cultures (group B streptococcus, two; *Escherichia coli*, four; *Bacteroides fragilis*, one). An additional six samples had positive amniotic fluid cultures but neg-

Table IV. Comparison of bacterial cultures and inhibition of leukocyte esterase activity

	n	*Leukocyte esterase activity* (range)*	*Ebelactone* (range)*
Uninfected	90	2.8-7.0	0.6-1.4
Infected	51	17.0-19.6	
Positive cultures	41		
Aerobic gram-positive			
Group B streptococcus	6	17.2-18.6	17.1-18.3
Enterococcus	10	17.0-19.3	17.1-19.3
Aerobic gram-negative			
E. coli	21	17.3-19.5	3.4-7.2
Anaerobic gram-negative			
B. fragilis	3	17.0-18.3	3.1-6.8
F. nucleatum	1	17.4	10.4

*Absorbance units.

ative Gram stains (group B streptococcus, two; *E. coli*, two; *B. fragilis*, two). Thirty-two samples were obtained from third-trimester amniotcenteses. Of these, 18 had positive results on both Gram stains and amniotic fluid cultures, and 10 had negative Gram stains but positive amniotic fluid cultures.

Forty-five of the 51 infected samples had positive leukocyte esterase activity (>12 enzyme activity units). Two of the six fluids with negative leukocyte esterase activity were second-trimester samples. One of these also had a positive amniotic fluid culture for group B streptococcus. The remaining four were from third-trimester samples. All four had negative amniotic fluid cultures.

The reliability of the diagnostic indices is shown in Table II. Gram stains of the amniotic fluid samples were not found to be highly sensitive (36.8%, second trimester; 56.3% third trimester). This finding is similar to a previous study in which we found Gram stains to be only 62% sensitive in third-trimester amniotic fluid samples.[5] The specificity of the Gram stains in second- and third-trimester fluids was 98.3% and 90.6%, respectively. In this study the sensitivity of amniotic fluid cultures was only 68.4% in the second-trimester samples and 87.5% in the third-trimester samples.

The various organisms identified on cultures are shown in Table III. When more than one organism was grown from an amniotic fluid sample, the infection was referred to as being caused by "mixed aerobic or anaerobic flora," and those samples were eliminated from analysis for this study. The paucity of anaerobic isolates obtained in this study may reflect bacterial fastidiousness or death or the limitations of the laboratory facilities that provided the results. There was one positive culture in the 90 uninfected samples. This was a third-trimester amniotic fluid sample in which group B Streptococcus ($>10^4$ cfu/ml) was isolated. There were 41 positive amniotic fluid cultures in the infected samples. As seen in the table, *E. coli* and enterococci were the two most common organisms identified in this group. However, if a positive culture was defined as one containing organisms $>10^2$ cfu/ml, then all 51 samples were positive. The organisms identified as $>10^2$ cfu/ml were as follows: group B streptococcus, one case; enterococcus, one case; streptococcus, one case; *E. coli*, two cases; Enterobacteriaceae, one case; *B. fragilis*, four cases.

The in vitro inhibition of the leukocyte esterase activity in the uninfected and infected samples with the nonspecific esterase inhibitor ebelactone is shown in Table I. The inhibition of the leukocyte esterase enzyme activity units ranged from 0 to 60% in both groups of samples. Further analysis of the comparison between the types of organisms cultured and the inhibition of the enzyme activity units by ebelactone is shown in Table IV. There were varying degrees of inhibition (range, 20% to 60%) in the uninfected samples. These same results were also found in the samples infected with gram-negative organisms (*E. coli, B. fragilis, Fusobacterium*). However, there was no inhibition of leukocyte esterase activity (range, 17.1 to 19.3) in the samples infected with gram-positive organisms (group B streptococcus, enterococci). These results were found to be statistically significant. The presence and ranges of inhibition were found to be similar in the samples with organisms $>10^2$ cfu/ml.

Comment

Despite the continuing seriousness of acute chorioamnionitis, which produces major morbidity in both mother and neonate, no optimal diagnostic findings currently exist. All attempts to improve our ability to diagnose this entity have been unsuccessful to date.

We previously reported on the reliability of measuring leukocyte esterase activity by a simple nonquantitative dipstick assay to diagnose intraamniotic infection in term and preterm gestations.[5,6] Because the results of the dipstick assay, like those of the aerobic and anaerobic cultures, can sometimes be misleading or equivocal we suggested the use of an in vitro spectropho-

tometric assay of esterase activity to eliminate some confusion.[4] This assay is rapid (<10 minutes) and simple to perform. It requires only a spectrophotometer and the determination of change in absorbance after the addition of substrate and inhibitor, both of which are easily available.

At present, even if clinical chorioamnionitis is reliably diagnosed, most standard protocols call for empiric choices of broad-spectrum antibiotics for immediate therapy. These choices are often based on preexisting theoretic knowledge of the pathogens that can cause such infections and not on the identification of specific organisms from the patient. This latter information is usually obtained much later (24 to 48 hours) when culture results are available. The results of our preliminary study suggest that the different bacteria that cause chorioamnionitis may elicit the production of different and specific esterases in infected amniotic fluid as shown by the differences in the in vitro inhibition of this activity by ebelactone. Thus it may be possible to identify the causative organisms in chorioamnionitis by measuring the absence, presence, and degree of in vitro leukocyte esterase inhibition by ebelactone. This would allow the formulation of timely and directed management decisions even before culture results are available.

REFERENCES

1. Gibbs RS, Castillo MS, Rodgers PJ. Management of acute chorioamnionitis. AM J OBSTET GYNECOL 1980;136:709.
2. Rindler-Ludwig R, Schmalzl F, Braunsteiner H. Esterases in human neutrophil granulocytes: evidence for their protease nature. Br J Haematol 1974;27:57.
3. Li CY, Lam KW, Yam LT. Esterases in human leukocytes. J Histochem Cytochem 1973;21:1.
4. Hoskins IA, Katz J, Ordorica SA, Young BK. Esterase activity in second and third trimester amniotic fluid: an indicator of chorioamnionitis. AM J OBSTET GYNECOL 1989; 161:1543.
5. Hoskins IA, Johnson TRB, Winkel CA. Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis. AM J OBSTET GYNECOL 1987;157:730.
6. Hoskins IA, Marks F, Ordorica SA, Young BK. Leukocyte esterase activity in amniotic fluid: normal values during pregnancy. Am J Perinatol 1990;7:130.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 7394. In the U.S., call toll-free: 1-800-325-4177, ext. 7394. Please allow six weeks for delivery.

Smoking and the antioxidant ascorbic acid: Plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women

Jayasri Basu, PhD, Magdy S. Mikhail, MD, Patricia H. Payraudeau, MD, Prabhudas R. Palan, PhD, and Seymour L. Romney, MD

Bronx, New York

The influence of cigarette smoking on the plasma, leukocyte, and cervicovaginal cell ascorbic acid levels in 46 healthy smokers and healthy nonsmokers was investigated. Coded peripheral venous blood and cervicovaginal lavage specimens obtained after informed consent were analyzed simultaneously for their ascorbic acid content. The findings suggest that smoking affects the levels and distribution of ascorbic acid. In smokers ($n = 16$), the number of exfoliated cervicovaginal epithelial cells and leukocyte ascorbic acid levels was significantly higher ($p < 0.01$, $p < 0.05$, respectively) compared with nonsmokers ($n = 30$). In addition, cervicovaginal cell ascorbic acid, plasma reduced and total ascorbic acid levels were significantly lower ($p < 0.001$, $p < 0.01$, $p < 0.01$, respectively). The exfoliated epithelial cell ascorbic acid levels in nonsmokers was fourfold greater than that of leukocytes. The implications of these findings within the context of free radical–induced cellular pertubations in smokers are discussed. (Am J Obstet Gynecol 1990;163:1948-52.)

Key words: Cigarette smoking, vitamin C, lavage technique, cell count, reduced ascorbic acid, total ascorbic acid, cervix cancer risk factors

The hypothesis that cigarette smoking is a causative factor in the development of cervical cancer has been evaluated by numerous investigators in case-control and cohort studies.[1-4] Epidemiologic studies suggest smoking to be an independent risk factor. The association between smoking and cancer of the cervix is seen to persist even after controlling for other known risk factors (e.g., multiple sexual partners, early age at first intercourse, or socioeconomic status).[5] Studies focused on the constituents of tobacco and its principal metabolites report that tobacco smoke contains mutagens and carcinogens and generates free radicals. These carcinogenic products are thought by some to act as initiators or promoters of the carcinogenic process.[6-8] Other studies revealed increases in mutagenic substances in cervical mucus of healthy women who are smokers.[9, 10] Although the deleterious effects of smoking are recognized and smoking is considered a risk factor for the development of cervical cancer,[1-11] the mechanism(s) whereby smoking exerts its carcinogenic effect(s) still remains obscure.

We have been investigating the role of nutrition in the pathogenesis of cervical cancer. A focus of our studies has been to delineate objective indices as a "risk profile" specifically correlated with cervical precancer states.[12-14] We previously determined the effect of smoking on the plasma levels of various nutrients in normal healthy women[15, 16] and those with cancer of the cervix.[12-14] The effects of smoking and human papillomavirus on plasma ascorbic acid and β-carotene levels in women with abnormal Papanicolaou smears were examined.[17] The findings of this investigation focus attention on a number of significant interactions triggered by cigarette smoking in apparently normal healthy women. Significant alterations in both the concentrations and distribution of plasma, leukocyte, and cervicovaginal cell ascorbic acid levels were noted in smokers.

Material and methods

Subject recruitment. Normal healthy women who attended the Women's Health Center of the Bronx Municipal Hospital Center in New York City for routine visits between August 1988 and March 1990 were asked to participate in the study. The study protocol was approved by the institutional review board. Forty-six women with two consecutive negative Papanicolaou smears who had normal regular menstrual cycles of ap-

From the Department of Obstetrics and Gynecology, Albert Einstein College of Medicine.
Supported in part by the Friends of Gynecology Cancer Research, Albert Einstein College of Medicine, and a grant-in-aid from Hoffmann-La Roche, Inc., Nutley, New Jersey.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Jayasri Basu, PhD, Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, Ullmann Building, Room 111, 1300 Morris Park Ave., Bronx, NY 10461.
6/6/24753

proximately 28 days, had no known gynecologic disorders, and were not using oral contraceptives or vitamin supplements were enrolled into the study. Subjects were not menstruating on the day samples were obtained. Peripheral venous blood and cervicovaginal lavage samples, collected by described methods,[14] were obtained from each participant with informed consent. Coded samples were transported to the laboratory promptly for ascorbic acid analyses. A self-administered study questionnaire including questions regarding age, parity, date of last menstrual period, contraceptive practices, and smoking history (the number of cigarettes smoked per day and years of smoking) was recorded. No dietary restrictions were imposed. However, on the morning samples were obtained the breakfast intake before the clinic visit was noted.

Analyses of ascorbic acid. Blood samples were collected in heparinized tubes. Plasma, leukocyte, and cervicovaginal cell ascorbic acid levels were determined promptly the same day the samples were collected without knowledge of the women's smoking or dietary status. Plasma was separated by centrifugation. Equal amounts of 0.9% sodium chloride were added to each tube and the leukocytes were removed by Ficoll method.[18] The total Ficoll layer was collected and centrifuged to obtain the leukocyte pellet. A known volume of the cervicovaginal lavage specimen was centrifuged to obtain a cervicovaginal cell pellet. To each of the known volumes of plasma, leukocyte, and cervicovaginal cell samples, 10% trichloroacetic acid was added. The plasma samples were thoroughly mixed. Leukocyte and cervicovaginal cell samples were additionally homogenized with a Polytron homogenizer. Each of the tubes containing the various samples was immersed in ice for 10 minutes and then centrifuged. The acidified supernatants were used to determine the levels of reduced and total ascorbic acid spectrophotometrically.[12]

Each assay was done in duplicate. Standard samples for both reduced and total ascorbic acid were analyzed simultaneously each day. The daily variation in standard ascorbic acid assays was less than 1%. The variation between duplicate samples, run simultaneously, was also less than 1%. Plasma reduced and total ascorbic acid levels are expressed as mg/dl. Leukocyte and cervicovaginal cell total ascorbic acid levels are expressed as micrograms per 10^6 cells. The lower limits of detection for the spectrophotometric methods for plasma reduced and total, leukocyte, and cervicovaginal total ascorbic acid levels were 50 mg/ml, and 0.01 $\mu g/10^6$ cells, respectively.

Leukocyte and cervicovaginal cell count. To a known volume of leukocyte suspension after Ficoll separation, or a cervicovaginal cell suspension, an equal volume of 2% Trypan blue solution was added. Each of the mixtures was dispensed onto a Neubeuer Hemacytometer. The cells distributed in the four corner squares were counted. Cell counts are expressed as number of cells $\times 10^6$ per milliliter.

Table I. Age, leukocyte, and cervicovaginal cell counts of smokers versus nonsmoker women

Group	*Age (yr)*	*Leukocyte count ($\times 10^6$ cells/ml)*	*Cervicovaginal cell count ($\times 10^6$ cells/ml)*
Smokers	32.5 ± 7.2 (n = 16)	11.64 ± 9.03 (n = 16)	1.24 ± 1.27 (n = 16)
Nonsmokers	28.0 ± 6.3 (n = 30)	14.71 ± 5.80 (n = 22)	0.58 ± 0.41 (n = 30)
p Value*	NS	NS	<0.01

Figures in parentheses indicate the number of subjects. Results are mean ± SD. Smokers on an average smoked 12.9 ± 9.5 cigarettes per day for 11.8 ± 7.6 years.

*By Student's t test.

Statistical analyses. The study code was broken in March 1990. Student's t test was applied to determine any association between the various study parameters: plasma reduced ascorbic acid, plasma total ascorbic acid, leukocyte total ascorbic acid, cervicovaginal cell total ascorbic acid and cervicovaginal cell and leukocyte count and (1) smoking, (2) phase of the menstrual cycle (women with last menstrual period ≤14 or >14 days), (3) methods of birth control (women not using any method versus those using barrier methods of birth control), and (4) breakfast intake (women who had or did not have breakfast on the day of sample collection).

Results

Sixteen of the 46 women in the study were smokers. Smokers on an average smoked 12.9 ± 9.5 cigarettes per day for 11.8 ± 7.6 years. The numbers of cigarettes smoked and the years of smoking varied from 2 to 35 per day and 4 to 25 years, respectively. Blood samples of eight nonsmoker women were obtained in amounts inadequate for leukocyte separation. The number of leukocytes and cervicovaginal cells of both smoker and nonsmoker subjects ranged from 1.14 to 32.4 and 0.1 to 4.64 $\times 10^6$ cells per milliliter, respectively. The ages of study subjects ranged from 18 to 44 years. The plasma reduced and total ascorbic acid, and leukocyte and cervicovaginal total ascorbic acid levels of all women in the study ranged from 0.18 to 0.917 mg/dl, 0.261 to 1.054 mg/dl; 0.073 to 0.554 $\mu g/10^6$ cells, and 0.01 to 1.797 $\mu g/10^6$ cells, respectively.

Table I compares the mean age, leukocyte, and cervicovaginal cell counts in smoker versus nonsmoker women. A significantly higher number of exfoliated cervicovaginal epithelial cells was observed in smokers. The cervicovaginal cell count of the smokers ranged

Table II. Distribution of plasma reduced and total, and leukocyte and cervicovaginal cell total ascorbic acid concentrations of smokers versus nonsmoker women

Group	*Plasma reduced ascorbic acid (mg/dl)*	*Plasma total ascorbic acid (mg/dl)*	*Leukocyte total ascorbic acid ($\mu g/10^6$ cells)*	*Cervicovaginal cell total ascorbic acid ($\mu g/10^5$ cells)*
Smokers	0.441 ± 0.235 (n = 16)	0.557 ± 0.218 (n = 16)	0.249 ± 0.146 (n = 16)	0.350 ± 0.378 (n = 16)
Nonsmokers	0.610 ± 0.190 (n = 30)	0.707 ± 0.170 (n = 30)	0.197 ± 0.092 (n = 22)	0.828 ± 0.546 (n = 30)
p Value*	<0.01	<0.01	<0.05	<0.001

Figures in parentheses indicate the number of subjects. Results are mean ± SD. Smokers on an average smoked 12.9 ± 9.5 cigarettes per day for 11.8 ± 7.6 years.

*By Student's t test.

from 0.01 to 4.64 × 10^6 cells per milliliter (median, 0.71 × 10^6). The cell counts of the nonsmokers ranged from 0.16 to 1.96 × 10^6 cells per milliliter (median, 0.46 × 10^6). The mean age and leukocyte count of the two study groups were comparable.

Table II compares the reduced and total plasma ascorbic acid levels, leukocyte, cervicovaginal cell total ascorbic acid levels of smokers versus nonsmoker healthy women. In smokers plasma reduced and total cervicovaginal cell total ascorbic acid levels were significantly lower, but the leukocyte total ascorbic acid level was significantly higher compared with the nonsmoker group. Because of the small sample size of smokers, statistical analysis to determine the effect of the number of cigarettes smoked times duration of smoking on the various study parameters could not be evaluated. The plasma ascorbic acid values analyzed by two separate procedures were found to be highly correlated (R = 0.55, p = 0.0001) and of similar magnitude.

The breakfast intake of each subject attending the morning clinics was analyzed. A total of 70% of the study population did not have any breakfast. Of those who had breakfast, 17% had only a cup of coffee, and two of the nonsmokers and two smokers had orange juice. The remaining subjects reported eating either one of a combination of the following: egg, toast, hamburger, hotdog, or French fries. All parameters studies were found to be comparable among women who ate or did not eat breakfast on the day of the clinic visit.

None of the study subjects used oral contraceptives. Subjects were not using any methods or were using barrier methods of birth control; when compared between these two groups the various study parameters were not statistically different. With regard to the menstrual cycle, there were 25 subjects in the follicular phase and 21 subjects in the luteal phase. The number of leukocyte and cervicovaginal cell counts of women in the follicular and luteal phases of the menstrual cycles was comparable ($p > 0.05$). In addition, ascorbic acid levels in the plasma, leukocytes, and cervicovaginal cells were not statistically different between women in the follicular versus luteal phase of the menstrual cycle.

Comment

This is the first study to report the levels of ascorbic acid simultaneously determined in plasma, leukocytes, and cervicovaginal cells in normal healthy women. Several noteworthy observations are apparent: (1) The exfoliation of cervicovaginal epithelial cells in smokers is significantly increased; (2) compared with leukocytes, the ascorbic acid level of exfoliated epithelial cells in nonsmokers is slightly higher; (3) in smokers, the difference in leukocyte and cervicovaginal cell ascorbic acid levels is markedly diminished; (4) leukocyte ascorbic acid level in smokers is significantly higher than in nonsmokers; (5) both plasma reduced and total ascorbic acid levels in smokers are significantly lower. The timing of the peripheral venous blood sample and cervicovaginal lavage sample collection with respect to the menstrual cycle of recruited subjects did not have any effect on any study parameters, indicating that the changes observed in this study were independent of menstrual cycle variations. Similarly, neither breakfast intake of the volunteer participants nor the method of birth control practiced was found to influence any of the study parameters. Smoking was the only variable that emerged as an influence on both the levels of ascorbic acid and exfoliation of cervicovaginal cells. Two separate assay procedures were used to determine the plasma reduced and total ascorbic acid levels. The plasma vitamin concentrations were found to be highly correlated and of equal magnitude. This suggests that ascorbic acid remains predominantly in the reduced state in normal healthy women.

Cancer prevention as an achievable goal challenges cancer research and has attained high priority. There is a substantial association between deficiency of dietary constituents and the incidence of certain malignancies.[19-21] Specific essential nutrients are hypothesized to reduce the cancer risk by neutralizing mutagens and

carcinogens, by quenching free radicals generated from various sources, or by augmenting the immune response.[6-8, 22, 23] Dietary substances with these attributes are vitamins A, C, E, β-carotene, and selenium.[24]

Biochemical evaluation of nutritional status is more precise and objective than dietary, clinical, or anthropometric assessments. In earlier investigations we assessed both dietary intake and plasma ascorbic acid levels in high-risk populations for cervix cancer.[12, 25] The results of this study confirm our earlier observations of significantly lower plasma reduced and total ascorbic acid levels in smokers (Table II) and is consistent with other investigators.[15, 26, 27] In peripheral blood, ascorbic acid has been shown to be highly liable and is partitioned between plasma and leukocytes according to the host's metabolic demands.[28] The leukocyte content of vitamin C is greater than the concentration in most other tissues, including plasma.[29]

Polymorphonuclear leukocytes are reported to generate reactive oxidants during phagocytosis.[30] Cervicovaginal cells may be at increased risk of free radical exposure as a consequence of smoke-induced carcinogens present in cervical mucus, and/or human papillomavirus infection, or both. An unexpected finding of this investigation is the high ascorbic acid levels of exfoliated epithelial cells in nonsmokers: the concentration was slightly higher than that of the leukocytes. The cervicovaginal cell ascorbic acid levels in smokers, although significantly lower than in nonsmokers, however, are greater than the leukocyte levels (Table II). Hence we suggest that in normal healthy women the antioxidant nutrient ascorbic acid is preferentially accumulated in both the leukocytes and cervix, tissues that are increasingly at risk of exposure to free radical damage.

There are several reports of the immuno-enhancing potential of ascorbic acid. Ascorbic acid deficiency in humans has been associated with impaired ingestion of microorganisms by polymorphonuclear leukocytes. The defective phagocytosis has been corrected by the administration of vitamin C.[31] Supplementation of ascorbic acid in healthy males resulted in a statistically significant increase in the serum levels of IgA, IgM, and C3 complement.[32] An elevation in macrophage ascorbic acid levels in smokers has also been reported.[33]

It is now established that hydrocarbon carcinogens absorbed from the pulmonary system are transmitted through the circulation to distant tissues and organs in cigarette smokers.[34] Nicotine and cotinine are selectively accumulated in the cervical mucus against a serum concentration gradient.[35] It is also reported that particulates found in cigarette smoke induce the release of a macrophage-derived chemotactic factor that attracts polymorphonuclear leukocytes. During phagocytosis, collagenase, elastase, or both are released from neutrophils with a concomitant increase in the production of free radical, "superoxide anions."[31, 36]

The higher content of leukocyte ascorbic acid in smokers despite a concentration gradient of significantly lower plasma reduced and total ascorbic acid levels suggests a preferential accumulation of the vitamin (Table II). The increment implies a requirement of the essential micronutrient by the leukocytes in smokers, possibly to enhance the host's defense mechanism. However, the comparatively lower cervicovaginal cell ascorbic acid levels in smokers (Table II) may reflect the increased utilization of ascorbic acid by the cervical tissue, counteracting the injurious effects of smoke-induced carcinogens and oxidative pertubations.

In this study increased numbers of exfoliated cervicovaginal cells were harvested from the lavage samples of smokers (Table I). This result is consistent with our previously reported finding.[14] Accumulation of polymorphonuclear leukocytes and macrophages in cervical tissue with a concomitant increase in the release of elastase and collagenase during phagocytosis may be responsible for decreasing the cohesiveness of cervicovaginal epithelial cells in smokers, resulting in increased exfoliation. Alternatively, smoking may induce an acceleration in the mitotic activity of cervicovaginal epithelial cells and expedite the exfoliation of cervical epithelium. We hypothesize that in cigarette smokers, inadequate concentrations of ascorbic acid and other antioxidants in cervical tissue render it susceptible to free radical damage. If deficiencies of antixodant nutrients in conjunction with excess production of free radicals persists for a prolonged period of time, cellular damage may ensue. Such events occurring over time in association with an altered immune response, and in the presence of other cervix cancer risk factors, may be an important component of the oncogenic process.

REFERENCES

1. Stellman SD, Austin H, Wynder EL. Cervix cancer and cigarette smoking: a case controlled study. Am J Epidemiol 1980;111:383-8.
2. Trevathan E, Latde P, Webster LA, et al. Cigarette smoking and dysplasia and carcinoma in situ of the uterine cervix. JAMA 1983;250:499-502.
3. Greenberg ER, Vessey M, McPherson K, et al. Cigarette smoking and cancer of the uterine cervix. Br J Cancer 1985;51:139-41.
4. Lyon JL, Gardner JW, West DW, et al. Smoking and carcinoma in situ of the uterine cervix. Am J Public Health 1983;73:558-62.
5. Hellberg D, Valentin J, Nilsson S. Smoking and cervical intraepithelial neoplasia: an association independent of sexual and other risk factors? Acta Obstet Gynecol Scand 1986;65:625-31.
6. Halliwell B, Gutterridge JM, eds. Free radicals in biology and medicine. Oxford: Clarendon Press, 1985.
7. Levine SA, Kidd PM, eds. Autooxidant adaptation: its role in free radical pathology. San Leandro, California: Allergy Research Group, Biocurrents Division, 1985.

8. Pryor WA. Cigarette smoke and the involvement of free radicals in chemical carcinogenesis. Br J Cancer 1987; 55(suppl 8);19-23.
9. Sasson IM, Haley NJ, Hoffmann D, et al. Cigarette smoking and neoplasia of the uterine cervix: smoke constituents in cervical mucus. N Engl J Med 1985;312:315-6.
10. Holly EA, Petrakis NL, Friend NF, Sarles DL, Lee RE, Flander LB. Mutagenic mucus in the cervix of smokers. JNCI 1986;76:983-6.
11. Brinton LA, Schairer C, Haenszel W, et al. Cigarette smoking and invasive cervical cancer. JAMA 1986;255: 3265-9.
12. Romney SL, Basu J, Vermund SH, Palan PR, DuttaGupta C. Plama reduced and total ascorbic acid in human uterine cervix dysplasias and cancer. Ann N Y Acad Sci 1987;498: 132-43.
13. Palan PR, Mikhail M, Romney SL. Decrease β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs. Am J Obstet Gynecol 1989;161:1649-52.
14. Basu J, Mikhail MS, Goldberg GL, Palan PR, Romney SL. Measurements of ascorbic acid and glutathione in exfoliated cervicovaginal epithelial cells of smokers and women with cervical dysplasias. Gynecol Obstet Invest 1990;30:48-51.
15. Basu J, Vermund SH, Mikhail M, Palan PR, Romney SL. Plasma reduced and total ascorbic acid in healthy women: effects of smoking and oral contraception. Contraception 1989;39:85-93.
16. Palan PR, Romney SL, Vermund SH, Mikhail M, Basu J. Effect of smoking and oral contraception on plasma β-carotene levels in healthy women. Am J Obstet Gynecol 1989;161:881-5.
17. Basu J, Palan PR, Vermund SH, Goldberg G, Burk RD, Romney SL. Plasma ascorbic acid and β-carotene levels in women evaluated for HPV infection, smoking and cervix dysplasia. Cancer Detect Prev [In press].
18. Fotino M, Merson EJ, Allen FH. Micromethod for rapid separation of lymphocytes from peripheral blood. Ann Clin Lab Sci 1971;1:131-3.
19. National Research Council Committee on Diet, Nutrition and Assembly of Life Sciences. Diet, nutrition and cancer. Washington DC: National Academic Press, 1982.
20. Connett JE, Kuller LH, Kjelsberg MO, et al. Relationship between carotenoid and cancer: the Multiple Risk Factor Intervention Trial (MRFIT) study. Cancer 1989;64:126-34.
21. Wald NJ, Boreham J, Hayward JL, Bulbrook RD. Plasma retinol, β-carotene and vitamin E levels in relation in future risk of breast cancer. Br J Cancer 1984;49:321-4.
22. Oshima H, Bartsch H. The influence of vitamin C on the in vivo formation of nitrosamines. In: Counsell JM, Hornig DH, eds. Vitamin C (ascorbic acid). London: Applied Science, 1982:215-24.
23. Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1987;1: 441-5.
24. Das UN. Nutrients, essential fatty acids and prostaglandins interact to augment immune responses and prevent genetic damage and cancer. Nutrition 1989;5:106-10.
25. Wassertheil-Smoller SS, Romney SL, Wylie-Rosett, et al. Dietary vitamin C and uterine cervical dysplasia. Am J Epidemiol 1981;114:714-24.
26. Smith JL, Hodges RE. Serum levels of vitamin C in relation to dietary and supplemental intake of vitamin C in smokers and nonsmokers. Ann N Y Acad Sci 1986; 498:144-51.
27. Keith RE, Mossholder SB. Ascorbic acid status of smoking and nonsmoking adolescent females. Int J Vitam Nutr Res 1986;56:363-6.
28. Loh HS. The relationship between dietary ascorbic acid intake and buffy coat and plasma ascorbic acid concentrations at different ages. Int J Vitam Nutr Res 1972;4: 80-5.
29. Loh HS, Wilson CWM. Relationship between leucocyte and plasma ascorbic acid concentrations. Br Med J 1971;3:733-5.
30. Baehner RL, Boxer LA, Allen JM, Davis J. Autooxidation as a basis for altered function by polymorphonuclear leukocytes. Blood 1977;50:327-35.
31. Anderson R. Effects of ascorbate on leucocytes. II. Effects of ascorbic acid and calcium and sodium ascorbate on neutrophil phagocytosis and post-phagocytic metabolic activity. S Afr Med J 1979;56:401-4.
32. Prinz W, Bortz R, Bergin B, Hersch M. The effect of ascorbic acid supplementation on some parameters of the human immunological defence system. Int J Vitam Nutr Res 1977;47:248-57.
33. McGowan SE, Parenti CM, Hoidal JR, Niewoehner DE. Ascorbic acid content and accumulation by alveolar macrophages from cigarette smokers and non-smokers. J Lab Clin Med 1984;104:127-34.
34. Schiffman MH, Haley NJ, Felton JS, et al. Biochemical epidemiology of cervical neoplasia: measuring cigarette smoke constituents in the cervix. Cancer Res 1987;47: 3886-8.
35. Hellberg D, Nilsson S, Haley NJ, Hoffman D, Wynder E. Smoking and cervical intraepithelial neoplasia: nicotine and cotinine in serum and cervical mucus in smokers and nonsmokers. Am J Obstet Gynecol 1988;158:910-3.
36. Hoidal JR, Niewoehner DE. Lung phagocyte recruitment and metabolic alterations induced by cigarette smoke in humans and in hamsters. Am Rev Respir Dis 1982; 126:548-52.

Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women

Denise Lee Cassidenti, MD, Ariel G. Vijod, MS, Marcela A. Vijod, BS, Frank Z. Stanczyk, PhD, and Rogerio A. Lobo, MD

Los Angeles, California

Because smoking is associated with an increased risk for osteoporosis, yet a decreased risk of endometrial carcinoma, a state of relative hypoestrogenism induced by smoking has been suggested. However, because previous data are unclear and do not reflect current trends in smoking intensity and estrogen prescriptions, we examined the estrogen profiles of postmenopausal women, by smoking status, both before and after oral micronized estradiol. Baseline levels of estrone, estradiol, estrone sulfate, and estrone glucuronide were similar in nonsmokers and smokers, but unbound (non-sex-hormone-binding-globulin–bound) estradiol was significantly lower in smoking women ($p < 0.05$) and sex-hormone-binding-globulin–binding capacity was higher ($p < 0.001$). After 1 or 2 mg of micronized estradiol, estrone and estradiol serum profiles were similar but unbound estradiol was significantly lower in women who were smokers ($p < 0.05$). Serum estrone glucuronide rose with treatment but was indistinguishable in nonsmokers and smokers. However, maximum changes in serum estrone sulfate were greater in smokers after administration of estrogen, suggesting a hepatic effect. Urinary estrone glucuronide levels increased after 8 hours of oral estrogen but were similar in nonsmokers and smokers with the two doses. It appears that even moderate smoking, as studied here, induces significant changes in hepatic estrogen metabolism and is best reflected by alterations in serum estrone sulfate and sex-hormone-binding-globulin–binding capacity that result in decreased serum unbound estradiol. However, these changes do not appear to require increasing the estrogen dosage to achieve physiologic levels of estrogen in postmenopausal smokers. (Am J Obstet Gynecol 1990;163:1953-60.)

Key words: Smoking, estrogen status, postmenopausal women, pharmacokinetic profiles

Cigarette smoking has been associated with an increased risk of osteoporosis[1] but a decreased risk of endometrial cancer[2] in postmenopausal women. MacMahon et al.[3] first reported decreased urinary estrogen levels in the luteal phase of premenopausal women who were smokers. Thus it has been suggested that smoking causes a relative hypoestrogenism that explains the increased risk of osteoporosis but decreased risk of endometrial cancer.

Jensen et al.[4,5] attempted to address the question directly and have reported lower estrone levels in smoking postmenopausal women after they received hormonal replacement. However, the baseline estrogen status of nonsmokers and smokers was not significantly different. Moreover, the women in this study received a mixture of sex steroids, including a progestin, and the effects were most marked only in women who received high doses of estrogen.

Because it appeared likely that any effects of smoking on estrogen status would be related to the amount and intensity of smoking, we thought it was important to examine this issue in women who were only moderate smokers to establish the clinical relevance of this association in our patient population. To study this issue and after review of the previous studies, we reexamined this issue and sought to determine: (1) the baseline estrogen status of postmenopausal women who smoke, (2) estrogen levels after smoking in women who ingest only moderate doses of estradiol, (3) the possible mechanisms explaining the postulated altered estrogen metabolism in smokers, and (4) whether an increased dose of estrogen would be required in smokers to achieve physiologic hormone replacement.

Material and methods

Subjects. Volunteers were 25 healthy postmenopausal women who had not received hormonal replacement for at least 2 weeks. All women had been postmenopausal for at least 2 years before the study. The women were healthy and had no history of cancer or chronic illnesses. Twelve women were nonsmokers, and 13 women were long-term smokers who reported

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Southern California School of Medicine.

Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.

Reprint requests: Rogerio A. Lobo, MD, Department of Obstetrics and Gynecology, Women's Hospital, Room 1M2, LAC/USC Medical Center, 1240 North Mission Road, Los Angeles, CA 90033.

6/6/24779

Table I. Characteristics of patient population

	n	*Age (yr, mean ± SE)*	*Ponderal index (mean ± SE)*
Estrace 1 mg			
Nonsmokers	9	58.4 ± 2.2	11.6 ± 0.1*
Smokers	10	58.7 ± 1.6	12.2 ± 0.1
Estrace 2 mg			
Nonsmokers	3	56.7 ± 3.3	11.7 ± 0.1*
Smokers	3	58.7 ± 2.0	12.5 ± 0.1

*$p < 0.01$.

smoking an average of 11.6 ± 1.4 cigarettes per day (range, 6 to 20 cigarettes per day).

Protocol. All patients were studied at 8 AM after eating a light breakfast to increase the absorption of the oral micronized estradiol. The women (nonsmokers and smokers) were randomly assigned to receive either 1 or 2 mg of micronized estradiol (Estrace, Mead Johnson, Evansville). Smokers were instructed to smoke a cigarette of their own brand immediately after ingesting the micronized estradiol and then continue to smoke at normal intervals during the remaining 8 hours. Blood samples were drawn at baseline, 1, 2, 4, and 8 hours after micronized estradiol was ingested to determine the acute hepatic effects and pharmacokinetic profiles of oral ingestion. A random urine sample was collected at the start of the study followed by an 8-hour collection to assess urinary excretion. All urine samples were immediately stored at −20° C. Sera samples obtained were separated and stored at −20° C until assayed. All samples were assayed for estrone, estradiol, sex-hormone-binding-globulin–binding capacity, unbound (non-sex-hormone-binding-globulin–bound) estradiol, and the serum estrogen conjugates estrone glucuronide and estrone sulfate, which reflect hepatic metabolism. Urine was analyzed for estrone glucuronide and creatinine levels.

Assays. The assays for estradiol, sex-hormone-binding-globulin–binding capacity, and unbound estradiol have been previously validated and described.[6-9] For the purposes of this study, these assay systems were developed and validated. Estrone was measured by means of a specific estrone radioimmunoassay (RIA) that does not require a chromatographic step before quantitation. To 1 ml of serum, approximately 1000 dpm of tritiated estrone (DuPont NEN Research Products, Boston; specific activity, 42 Ci/mmol) in 0.1 ml of assay buffer [0.1 mol/L sodium phosphate, pH 7.0; 0.15 mol/L sodium chloride; 0.1% (wt/vol) Knox unflavored gelatin; 0.1% (wt/vol) sodium azide] is added as an internal standard to monitor procedural losses and incubated at 37° C for 30 minutes. The serum is extracted twice with 5 ml of ethyl acetate/hexane (4 : 6, vol/vol), and the combined organic phases are evaporated under nitrogen. The residue is reconstituted in 10% ethanol in assay buffer (vol/vol). The RIA is performed with a highly specific antiserum (Pantex, Santa Monica) directly against estrone. The antiserum exhibited a 0.14% cross-reaction with estradiol.

These reliability criteria were determined for the estrone RIA. The sensitivity of the assay (defined as the smallest amount of estrone per RIA tube that reduces the number of counts per minute of tritiated estrone bound at zero mass by 2 SDs) was 3.9 pg per tube or 15 pg/ml. Accuracy was determined by adding known amounts of authentic estrone to pooled serum. Linear regression analysis performed to compare estrone values measured in pools versus the amount of estrone added revealed a correlation coefficient of 0.99. Parallel experiments comparing the current assay with the previous validated estrone assay, requiring column chromatography,[10] were performed and showed equivalent values with an average difference of 5 pg/ml. The interassay coefficient of variation for estrone was 14%.

Estrone glucuronide was measured in an aliquot of 0.6 ml of serum combined with an internal standard of approximately 100 dpm of tritiated estrone glucuronide (Amersham Laboratories, Buckinghamshire, England; specific activity, 12.6 Ci/mmol) dissolved in 0.1 ml of 0.2 mol/L sodium phosphate buffer, pH 6.8, to monitor procedural losses and incubated at 37° C for 30 minutes. After extraction of unconjugated steroids, 2 ml of the phosphate buffer is added to the aqueous layer. To each sample 500 units of β-glucuronidase, type VIIA from *Escherichia coli* (Sigma Chemical Co., St. Louis) in 0.1 ml of the phosphate buffer is added and incubated overnight at 37° C. The hydrolyzed estrogens are extracted twice with 5 ml of ethyl acetate/hexane (4 : 6, vol/vol). After evaporating the organic solvents, the extract is reconstituted and the estrone is measured as described above. All values were corrected for molecular weight.

The following reliability criteria were determined for the estrone glucuronide RIA. The sensitivity of the assay was 45 pg/ml. Accuracy experiments revealed a correlation coefficient of 0.99. The specificity of the β-glucuronidase for conjugated estrone was 0.27%. The interassay coefficient of variation was 9.3%.

Estrone sulfate was measured on the basis of a modification of the procedure described by Platia et al.[11] To a 0.2 ml aliquot of serum, approximately 1000 dpm of tritiated estrone sulfate (DuPont NEN Research Products; specific activity, 60 Ci/mmol) in 0.1 ml of sodium acetate buffer, pH 5, is added as an internal standard to monitor procedural losses and is incubated at 37° C for 30 minutes. The samples are extracted twice with 5 ml of ethyl acetate/hexane (4 : 6, vol/vol) to remove unconjugated steroids. The aqueous phases are combined and deproteinated with 2 ml of methanol. After

Table II. Baseline estrogen status of nonsmoking and smoking postmenopausal women

	Estrone (pg/ml)	*Estradiol (pg/ml)*	*Unbound estradiol (pg/ml)*	*Estrone glucuronide (ng/ml)*	*Estrone sulfate (ng/ml)*
Nonsmokers ($N = 12$)	64 ± 8	19 ± 3	8.6 ± 1.4	0.193 ± 0.03	1.58 ± 0.25
Smokers ($N = 13$)	63 ± 6	14 ± 1	4.9 ± 0.6*	0.199 ± 0.03	1.65 ± 0.21

*$p < 0.01$.

the mixture is spun in a vortex, the protein pellet is removed by centrifugation, and the methanol is evaporated under nitrogen at 40° C. The residue is reconstituted in 2 ml of the acetate buffer, and 6.7 units of arylsulfatase type VIII from abalone entrails (Sigma Chemical Co.) is added, followed by an overnight incubation at 37° C. The hydrolyzed estrogens are extracted twice with 5 ml of ethyl acetate/hexane (4:6, vol/vol) mixture. After the organic solvents are evaporated, the extract is reconstituted and the estrone is measured as described earlier. All values were corrected for molecular weight and for estrone glucuronide procedural losses.

The following reliability criteria were determined for estrone sulfate RIA. The sensitivity of the assay was 160 pg/ml. The average efficiency of hydrolysis was 85% with an overall recovery of 71%. Parallelism was established by serial dilution of pooled serum. The resulting curve was parallel to the corresponding standard curve. Specificity of the arylsulfatase enzyme for estrone glucuronide was 8%. The interassay coefficient of variation was 11%. Assays for urinary estrone glucuronide and creatinine were performed as previously described.[12]

Statistical analyses were done between groups with paired and unpaired t tests and logarithmic transformation. All data are reported as mean ± SE.

Results

Table I depicts the characteristics of our patients. Body habitus is reported by the ponderal index, which is height (inches) divided by the cube root of the weight (pounds). Patients receiving Estrace 1 or 2 mg were matched for age. However, on the basis of ponderal indices, the nonsmokers were significantly heavier than the smokers in both groups ($p < 0.01$).

Baseline estrogen status of nonsmokers ($n = 12$) and smokers ($n = 13$) is depicted in Table II. There were no differences in levels of estrone, estradiol, estrone glucuronide, or estrone sulfate. However, baseline levels of unbound estradiol were significantly lower in smokers than in nonsmokers (4.97 ± 0.62 vs 8.56 ± 1.4 pg/ml, $p < 0.01$).

With Estrace 1 mg, nonsmokers and smokers had similar estradiol and estrone levels for 8 hours after ingestion (Fig. 1). Peak levels averaged 200 pg/ml for estrone and 40 pg/ml for estradiol in both groups. However, levels of unbound estradiol, although similar in nonsmokers and smokers after 1 hour, were significantly lower in smokers at 8 hours (13.3 ± 1.4 vs 25.9 ± 4.1 pg/ml, $p < 0.02$). Serum estrone glucuronide peaked at two hours and decreased rapidly thereafter. Conversely, estrone sulfate showed a gradual rise that reached maximum levels at 4 hours (20 ng/ml) and these levels were sustained thereafter. No statistical differences were observed for these serum conjugates in nonsmokers and smokers.

In patients who received Estrace 2 mg, smokers had higher levels of estrone compared with nonsmokers at 1 hour (200 ± 34.6 vs 99.3 ± 9.1 pg/ml, $p < 0.05$) (Fig. 2). The same trend was observed with estradiol but was not statistically significant. Over the entire 8 hours, values of estrone and estradiol were not significantly different in nonsmokers and smokers and peaked at levels of 350 and 50 pg/ml, respectively. Unbound estradiol was not significantly different at baseline but was significantly lower in smokers than in nonsmokers, both at 4 hours (14.9 ± 1.5 vs 29.8 ± 4.6 pg/ml, $p < 0.05$) and at 8 hours (16.9 ± 1.5 vs 36.9 ± 11.5 pg/ml, $p < 0.05$). Serum estrone glucuronide levels showed a rapid increase in smokers but were not statistically different from responses of nonsmokers. Serum estrone sulfate levels were also higher in smokers than in nonsmokers, but these values were only of borderline significance ($p < 0.06$).

Sex-hormone-binding-globulin–binding capacity was significantly higher in smokers compared with nonsmokers in both treatment groups: Estrace 1 mg (smokers vs nonsmokers, 136.7 ± 8.2 vs 89.8 ± 8.2 nmol/L; $p < 0.0001$) and Estrace 2 mg (smokers vs nonsmokers, 191.3 ± 13.2 vs 61.6 ± 8.3 nmol/L, $p < 0.0001$) (Fig. 3). This 35% to 68% difference in sex-hormone-binding-globulin–binding capacity between smokers and nonsmokers could not be accounted for solely on the basis of body mass because there was only a 5% to 7% difference in ponderal index between the two groups. As expected, there were no changes with 8 hours of treatment (data not shown).

To better analyze estrogen metabolism, we examined the maximum changes in the hepatic markers, serum

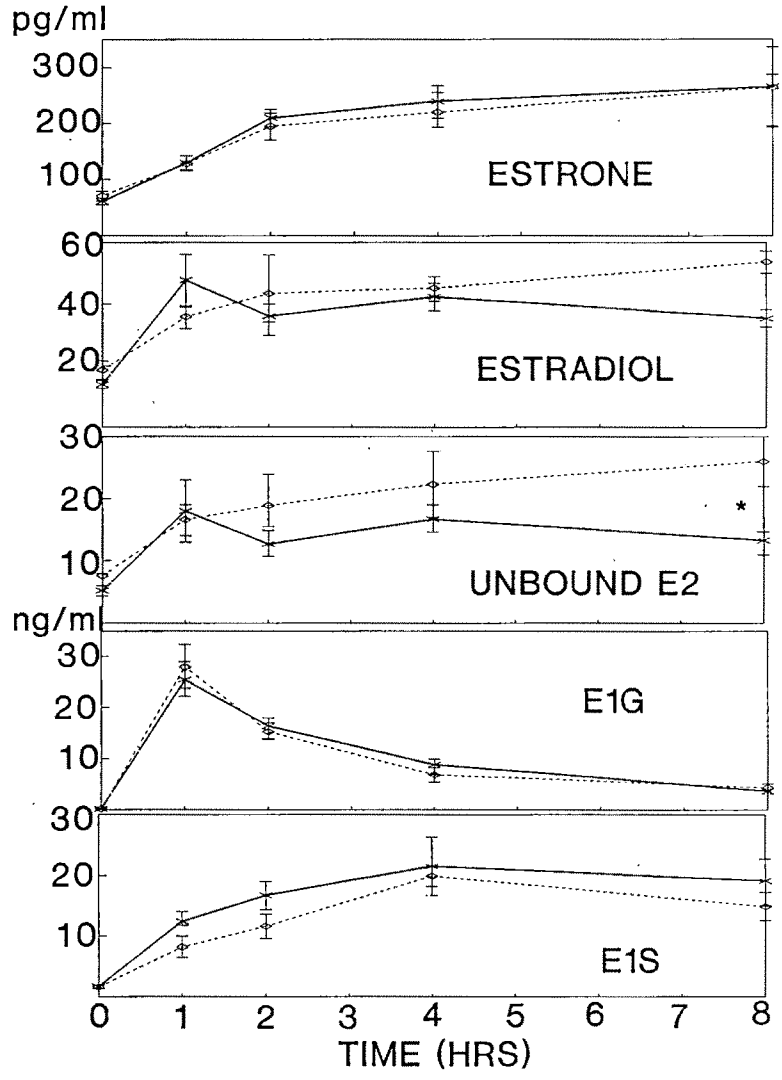


Fig. 1. Pharmacokinetic profiles in women receiving Estrace 1 mg. Nonsmokers *(dotted line)* are compared with smokers *(solid line)*. *Asterisk*, $p < 0.02$.

estrone glucuronide and estrone sulfate, from baseline to peak. When the groups receiving Estrace 1 mg and 2 mg were compared, the maximum changes in levels of estrone glucuronide were not statistically different in nonsmokers and smokers (Fig. 4). However, a trend was observed with Estrace 1 mg; the maximum changes in estrone sulfate were higher in smokers than in nonsmokers but was not statistically significant. In the 2 mg group, this difference in estrone sulfate was statistically significant (24.4 ± 4.2 vs 8.7 ± 0.2; $p < 0.05$).

Concentrations of urinary estrone glucuronide (micrograms per gram of creatinine) in the patients are shown in Fig. 5. At baseline, urinary estrone glucuronide was virtually undetectable and was not different in nonsmokers and smokers. Eight hours after oral ingestion, estrone glucuronide levels rose significantly. With Estrace 1 mg, the values were similar for nonsmokers (729 ± 110 μg estrone glucuronide per gram of creatinine). With Estrace 2 mg, no statistical difference was observed between nonsmokers (1470.7 ± 264 μg estrone glucuronide per gram of creatinine) and smokers (1829.3 ± 855 μg estrone glucuronide per gram of creatinine).

Comment

There were no differences in baseline levels if estrone, estradiol, and the conjugates, estrone sulfate and estrone glucuronide, between untreated postmenopausal nonsmokers and smokers. However, baseline unbound estradiol was significantly lower in postmenopausal women who smoked. This could be firmly established only when all women participating in this study were evaluated together (both treatment groups at baseline). This finding is most likely the result of increased sex-hormone-binding-globulin–binding capacity in the women who smoked. Thus, even though our

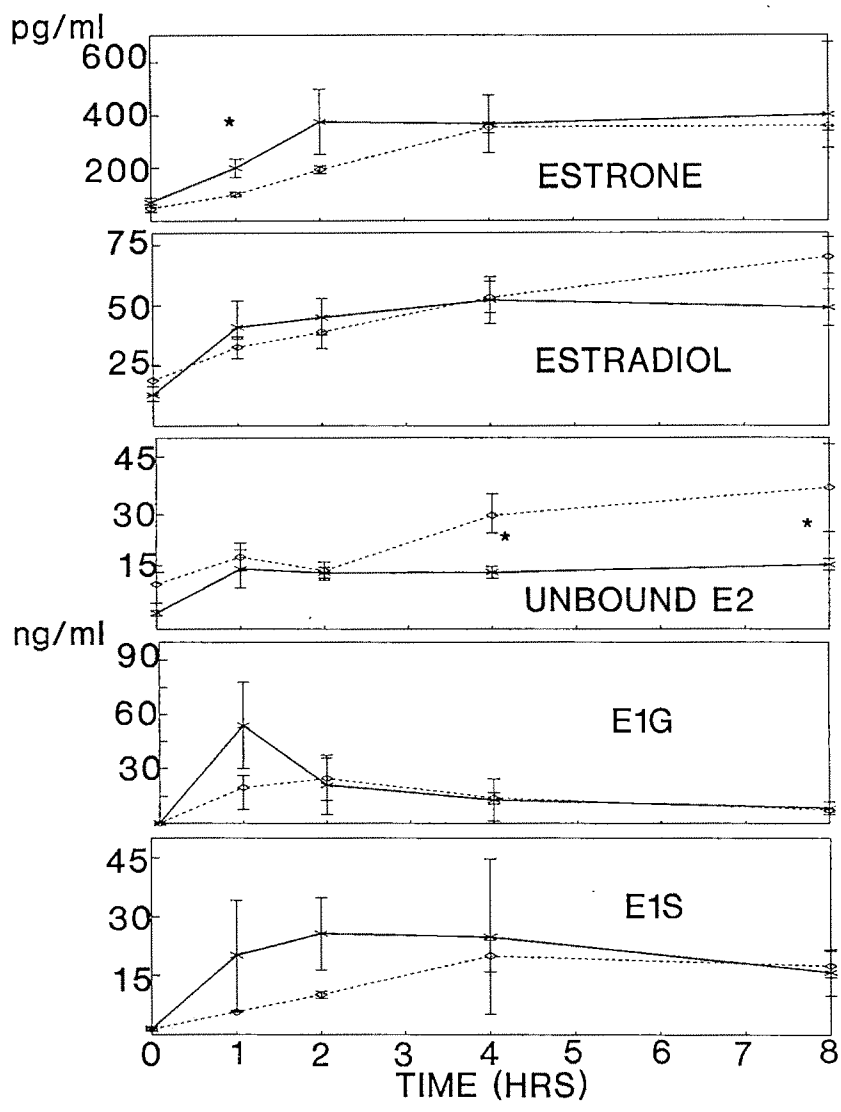


Fig. 2. Pharmacokinetic profiles in women receiving Estrace 2 mg. Nonsmokers *(dotted line)* are compared with smokers *(solid line)*. *Asterisk*, $p < 0.05$.

patients were only moderate smokers (smoking an average of 11.6 ± 1.4 cigarettes per day), the findings in estrogen status were of significance. This degree of smoking is reflective of current smoking trends in California.[13] Studies done by the National Health Interview Survey over the last 10 years have shown a steady decline in smoking prevalence in the United States from 36.7% in 1974 to 30.4% in 1985. A decline was also seen when men and women were analyzed separately. Women showed a steady decline in smoking of 0.33% per year.[14]

With use of 1 mg oral micronized estradiol, levels of estrone and estradiol were not significantly different. Increasing the dose of estradiol to 2 mg did not significantly change these profiles. In comparison with our results, Anderson et al.,[15] who administered oral estradiol valerate, showed similar peak levels of estrone and estradiol. However, maximum levels in this study were seen at 4 hours and were noted to decline at 6 hours, whereas in our study estrone and estradiol levels were sustained. Although our levels of estrone sulfate are comparable to those previously reported,[15] our values for serum estrone glucuronide with oral estrogen are twofold to threefold higher than values reported by Siddle and Whitehead.[16] These differences in the measurements and pharmacokinetics of estrogen may be related to assay differences and the absorption characteristics of micronized estradiol.

Unbound estradiol was significantly lower in smokers after oral Estrace administration. This was more marked with Estrace 2 mg where significant changes occurred at 4 and 8 hours. The data on unbound estradiol, being reflective of biologically available estradiol, suggest that smokers have less biologically avail-

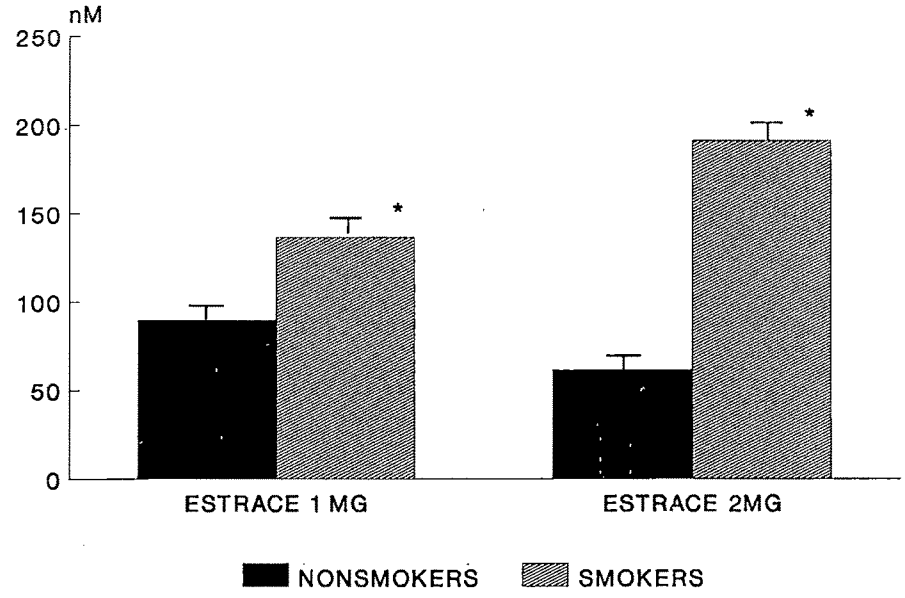


Fig. 3. Sex-hormone-binding-globulin–binding capacity in nonsmokers compared with smokers in the two treatment groups. *Asterisk*, $p < 0.0001$.

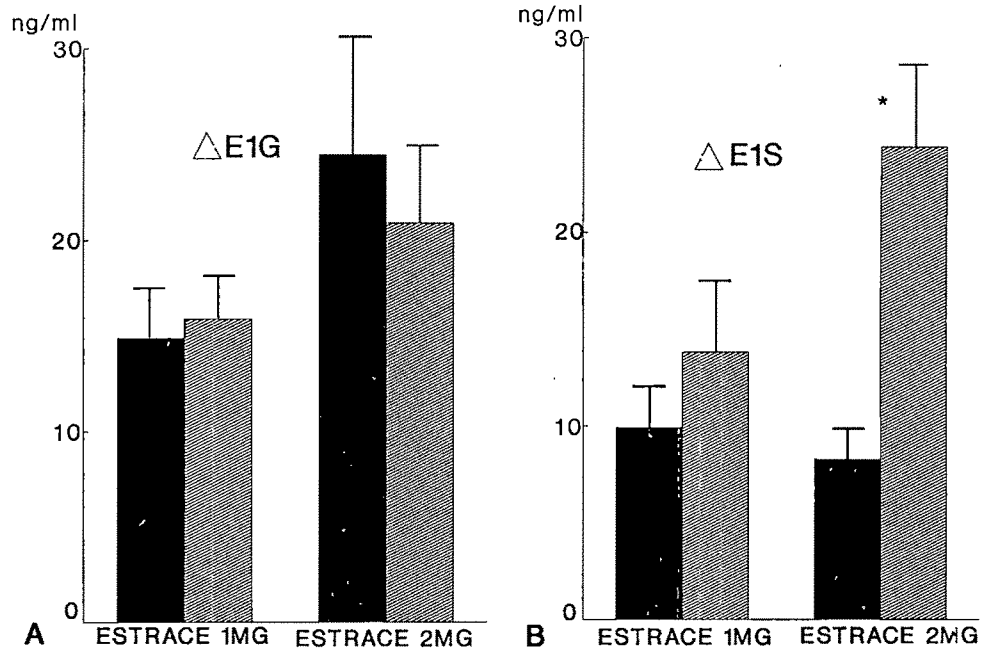


Fig. 4. Maximum changes from baseline in hepatic markers of estrogen metabolism with smoking. **A**, nonsmokers versus smokers. **B**, Estrone sulfate, nonsmokers versus smokers. *Black bars*, nonsmokers; *hatched bars*, smokers. *Asterisk*, $p < 0.05$.

able estradiol than nonsmokers. This supports data that implicated elevated free estradiol as a risk factor for endometrial cancer[17] and may explain the decreased incidence of endometrial carcinoma seen in smokers. The presence of lower biologically available estradiol also supports evidence that places smokers at a higher risk for osteoporosis.

Body weight is inversely related to sex-hormone-binding-globulin–binding capacity. Although groups matched for weight in this study would have been ideal, it is well known that smokers have lower body weights than nonsmokers and matching would have been difficult. Despite the differences in body weight, the large difference in sex-hormone-binding-globulin–binding

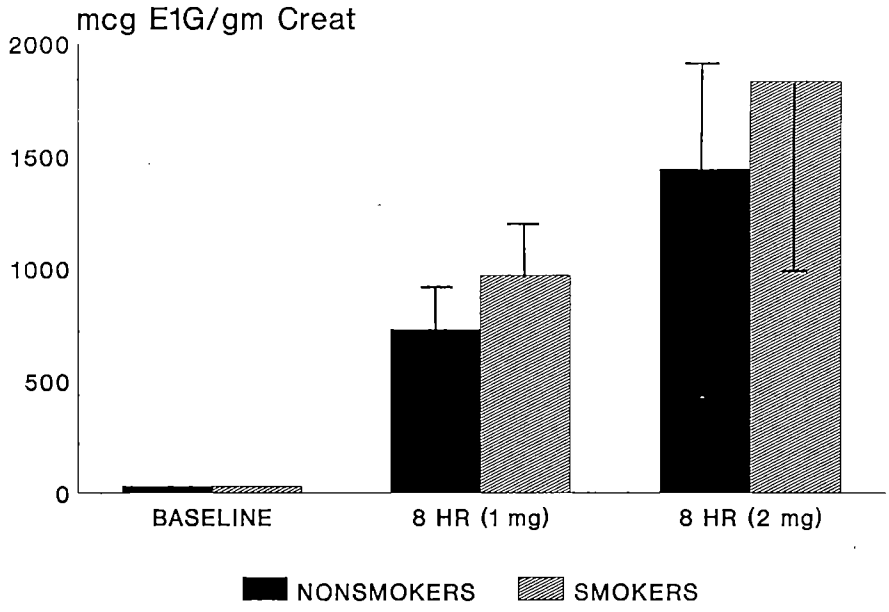


Fig. 5. Urinary estrone glucuronide in nonsmokers and smokers at baseline and in an 8-hour collection after administration of Estrace 1 or 2 mg.

capacity between smokers and nonsmokers is greater than what would be expected solely on the basis of increased body weight alone. Linear regression analysis showed only a loose correlation between sex-hormone-binding-globulin–binding capacity to ponderal index ($r = 0.43, p < 0.04$). This alone does not fully explain our data since baseline levels of unbound estradiol were similar in smokers and nonsmokers. Only after oral estrogen ingestion did unbound estradiol levels change. Sex-hormone-binding-globulin is known not to increase over this short period of time (8 hours).

Our data are similar to that of Jensen et al.,[4, 5] in which baseline levels of serum estrone and estradiol in untreated postmenopausal women were not statistically different. In this Danish study, differences in both estrone and estradiol levels were only observed when high doses of estrogen (4 mg of estradiol) were used. In the same study, the women received a mixture of sex steroids, including a progestin, norethisterone acetate, that may have influenced hepatic clearance.

Serum estrogen conjugates, as markers of hepatic metabolism, are important for the complete evaluation of the pharmacokinetic profiles of our patients. Smoking appears to increase hepatic metabolism in a dose-related manner, resulting predominantly in increased serum estrone sulfate. Because estrone glucuronide and estrone sulfate are hepatic products and significant changes in estrone sulfate were observed only with Estrace 2 mg and was of borderline significance with 1 mg, this suggested to us that the effect was dose-related. Several studies have examined changes in the estrogen conjugates estrone sulfate and estradiol sulfate after administration of various sex steroids.[18, 19] The rise in these conjugates has been shown to be dose-related[20] as we have demonstrated here. The relatively long half-life of estrone sulfate, because of its high affinity to albumin, resulted in higher levels that made changes easier to detect. Serum estrone glucuronide is cleared rapidly from the circulation and was not different between groups in this study.

Estrone glucuronide increased after administration of micronized estradiol but was cleared by the kidneys faster than estrone sulfate. No differences in the urinary levels of estrone glucuronide between the two groups were noted. Thus no direct effects of smoking on renal clearance could be demonstrated. We would have predicted that the rapid increase in hepatic metabolism would have also resulted in lowered estrone levels in addition to unbound estradiol, but we were unable to demonstrate this. Although this should be related to the intensity of smoking, no correlation could be found between the number of cigarettes smoked per day and the levels of estrone, estradiol or unbound estradiol.

Therefore we hypothesize that smoking increases hepatic metabolism leading to increases in sex-hormone-binding-globulin–binding capacity, estrone sulfate, and estrone glucuronide. The effects are most marked in the case of sex-hormone-binding-globulin–binding capacity. With estrogen treatment, changes in serum estrone sulfate were more marked than those of serum estrone glucuronide. The effects observed are related to the dose of estrogen ingested, with greater effects observed at higher doses. Only minmal differences in

estrone sulfate and estrone glucuronide were observed with Estrace 1 mg, and only when Estrace 2 mg was used were the observed effects significant.

Increased doses of estradiol would likely have resulted in more profound effects on estrogen metabolism than the doses studied here. However, because postmenopausal hormonal replacement usually does not exceed Estrace 2 mg, the effects of moderate smoking appear to be mild. Although differences do exist, on the basis of these data, these differences do not appear to be large enough to warrant increasing routine doses of estrogen replacement in postmenopausal women who smoke.

REFERENCES

1. Daniell HW. Osteoporosis of the slender smoker. Arch Intern Med 1976;136:298-304.
2. Lesko SM, Rosenberg L, Kaufman DW, et al. Cigarette smoking and the risk of endometrial cancer. N Engl J Med 1985;313:593-6.
3. MacMahon B, Trichopoulos D, Cole P, Brown J. Cigarette smoking and urinary estrogens. N Engl J Med 1982;307: 1062-5.
4. Jensen J, Christiansen C, Rodbro R. Cigarette smoking, serum estrogens, and bone losses during hormone-replacement therapy early after menopause. N Engl J Med 1985;313:973-5.
5. Jensen J, Christiansen C. Effects of smoking on serum lipoproteins and bone mineral content during postmenopausal hormone replacement therapy. Am J Obstet Gynecol 1988;159:820-5.
6. Stanczyk FZ, Shoupe D, Nunez V, Macias-Gonzales P, Vijod MA, Lobo RA. A randomized comparison of non-oral estradiol delivery in postmenopausal women. Am J Obstet Gynecol 1988;159:1540-6.
7. Rosner W. A simplified method for the quantitative determination of testosterone-estradiol binding globulin activity in human plasma. J Clin Endocrinol Metab 1972;34: 983-8.
8. Stumpf PG, Nakamura RM, Mishell DR Jr. Changes in physiologically free circulating estradiol and testosterone exposure to levonorgestrel. J Clin Endocrinol Metal 1981; 52:138-43.
9. Tremblay RR, Dube JY. Plasma concentrations of free and non-TeBG bound testosterone in women on oral contraceptives. Contraception 1974;10:599-605.
10. Goebelsmann U, Bernstein GS, Gale JA, Kletzky OA, Nakamura RM. Serum gonadotropin, testosterone, estradiol and estrone levels prior to and following bilateral vasectomy. In: Lepow IH, Crozier R, eds. Vasectomy: immunologic and pathophysiologic effects in animals and man. New York: Academic Press, 1979:165-81.
11. Platia MP, Fencl MD, Elking-Hirsch KE, Canick JA, Tulchinsky D. Estrone sulfatase activity in human brain and estrone sulfate levels in the normal menstrual cycle. Steroid Biochem 1984;21:237-41.
12. Stancyzk FZ, Miyakawa I, Goebelsmann U. Direct radioimmunoassay of urinary estrogen and pregnanediol glucuronides during the menstrual cycle. Am J Obstet Gynecol 1980;137:443-50.
13. Marcus AC, Shopland DR, Crane LA, Lynn WR. Prevalence of cigarette smoking in the United States: estimates from 1985 current population survey. JNCI 1989;81:409-14.
14. Fiore MC, Novotny TE, Pierce JP, Hatziandreu EJ, Patel KM, Davis RM. Trends in cigarette smoking in the United States. JAMA 1989;261:49-55.
15. Anderson ABM, Sklovsky E, Sayers L, Steele PA, Turnbull AC. Comparison of serum oestrogen concentrations in post-menopausal women taking oestrone sulphate and oestradiol. Br Med J 1978;1:140-2.
16. Siddle N, Whitehead M. Flexible prescribing of estrogens. Contemp Ob/Gyn 1983;22:137-66.
17. Nisker JA, Hammond GL, Davidson BJ, et al. Serum sex hormone-binding globulin capacity and the percentage of free estradiol in postmenopausal women with and without endometrial carcinoma. Am J Obstet Gynecol 1980;138: 637-42.
18. Aedo A-R, Sunden M, Landgren B-M, Diczfalusy E. Effect of orally administered oestrogens on circulating oestrogen profiles in postmenopausal women. Maturitas 1989;11:159-68.
19. Towobola OA, Crilly RC, Oakey RE. Oestrone sulphate in plasma from postmenopausal women and the effects of oestrogen and androgen therapy. Clin Endocrinol 1980;13:461-71.
20. Dada OA, Laumas V, Landgren B-M, Cekan SZ, Diczfalusy E. Effect of graded oral doses of oestradiol on circulating hormonal levels. Acta Endocrinol 1978;88:754-67.

Oxytocin and vasopressin binding sites in human and bovine ovaries

Anna-Riitta Fuchs, DSc, Oliver Behrens, MD, Hanns Helmer, MD, Annette Vangsted, MD, Marina Ivanisevic, MD, Jamie Grifo, MD, PhD, Ciro Barros, PhD, and Michael Fields, PhD

New York, New York, and Gainesville, Florida

Human ovarian tissue and bovine ovarian stroma and follicles bound tritiated oxytocin and tritiated arginine vasopressin with similar affinity, whereas bovine corpora lutea bound tritiated oxytocin only. Competition for the binding of tritiated oxytocin by various agonists and antagonists was suggestive of receptor function. The number of oxytocin binding sites varied cyclically in all tissues. In bovine ovarian stroma and corpora lutea the concentrations were lowest on day 14 and highest on days 17, 19, and 21 after ovulation, with a striking peak in the luteal concentration on day 19. In human ovarian tissues the concentrations also were highest in samples obtained in late luteal phase. In large follicles the concentration of oxytocin binding sites was highest on the day of estrus and lowest on day 7. In bovine ovary the number of arginine vasopressin binding sites was ~50% lower than oxytocin binding sites and the cyclic variations were not significant. Human ovarian tissue had similar numbers of oxytocin and arginine vasopressin binding sites. Because bovine ovaries produce oxytocin and argine vasopressin the results suggest a paracrine or autocrine role for these neuropeptides in luteolysis and ovulation. Although their synthesis in human ovaries is still controversial the presence of binding sites suggests a physiologic role in the regulation of human ovarian function as well. (AM J OBSTET GYNECOL 1990;163:1961-7.)

Key words: Oxytocin, vasopressin, receptors, ovaries, corpora lutea, follicles

Oxytocin has been found in the corpora lutea of many species including cow,[1, 2] sheep,[3] pig,[4] rat,[5] deer,[3] human,[6] and cynomolgus monkey.[7] It is produced by the granulosa-derived large luteal cells[4, 8] and periovulatory granulosa cells.[4, 9, 10] The ability of oxytocin to affect luteal function has been demonstrated in several species including the ruminants[11, 12] and primates.[13] In species in which the uterus is essential for the regulation of corpus luteum life span, oxytocin is thought to act on the endometrium to cause the release of prostaglandin $F_{2\alpha}$[14] ($PGF_{2\alpha}$), which in turn acts on the corpus luteum to cause luteolysis.[15, 16] In species in which the influence of the uterus on luteal life-span is not significant (e.g., the primates), oxytocin may have a paracrine role in the regulation of ovarian function, acting on ovarian tissues to release $PGF_{2\alpha}$, which then diffuses into the adjacent luteal cells to cause luteolysis.[17] To determine the validity of such a hypothesis, we examined the binding of oxytocin to ovarian tissues because the presence of receptors is a prerequisite for oxytocin action. Arginine vasopressin is produced in the ovaries of some species,[5] and specific binding sites for arginine vasopressin have been found in other tissues of the female reproductive tract.[18, 19] We therefore examined the tissues also for the presence of arginine vasopressin binding sites.

From the Department of Obstetrics and Gynecology, Cornell University Medical College, and the Department of Animal Science, University of Florida.

Supported in part by a grant from Warner Lambert Foundation, Deutsche Forschungsgemeinschaft, The Lundbeck Foundation, The Danish Medical Research Council, the Fulbright Foundation, and grant US-1160-86C from BARD, the United States–Israel Binational Agricultural Research and Development Fund, Florida Agricultural Experimental Station Journal no. R-01093.

Presented in part at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.

Reprint requests: Anna-Riitta Fuchs, DSc, Room 412, Department of Obstetrics and Gynecology, Cornell University Medical College, 1300 York Ave., New York, NY 10021.

6/6/24755

Material and methods

Human subjects. Ovarian tissue was obtained from nine premenopausal women who had operations for the following nonmalignant causes: ovarian cysts (three cases), endometriosis (three), benign ovarian tumor (one), fibroids of the uterus (one), and hydrosalpinx (one). The contralateral ovary was used in all instances but one, and the tissue was histologically normal in all instances. All patients gave their consent and the project was approved by the Institutional Human Rights Committee. The tissues were kept on ice until the pathologists had examined the ovarian tissue and cut a portion of macroscopically normal tissue without the capsule and corpora lutea. The tissue was then wrapped tightly in foil and frozen at −70° to −80° C until assayed. Because insufficient tissue was available from individual patients to permit saturation analysis, tissues were pooled according to the stage in menstrual cycle

when the operation was performed. This was determined from the menstrual history and verified by histologic examination of the endometrium. The following pools were obtained: 1, proliferative phase, days 6 and 7 (two pools); 2, midcycle, days 14 to 18 (two pools); 3, late luteal phase, days 19 to 28 (one pool).

Animals. Eighteen Hereford cows from the herd of the Animal Science Department of the University of Florida were killed on days 7, 14, 17, 19, and 21 after behavioral estrus (three cows each day). The tissues were removed as quickly as possible, the ovaries were freed of peritoneum, large follicles and corpora lutea were dissected, and the stroma, follicles, and corpora lutea were frozen separately and kept at −70° to −80° C until assayed.

Tissue preparation. The tissues were homogenized in 10 mmol/L Tris buffer containing 3 mmol/L ethylenediaminetetraacetic acid, 0.5 mmol/L dithiothreitol, and 0.002% of a protease inhibitor, phenylmethylsulfonyl fluoride (Sigma, St. Louis). Four 5-second pulses with a Polytron tissue grinder (Brinkman, Westbury, N.Y.) with 30-second intervals were used followed by four up-and-down strokes with a hand-held glass tissue grinder (Kontes, Vineland, N.J.). All steps were performed in an ice bath. A crude membrane preparation precipitated between 1000 *g* (10 minutes) and 160,000 *g* (30 minutes) at 4° C was used to maximize binding. The membrane preparations were washed repeatedly in 3 mmol/L ethylenediaminetetraacetic acid buffer to dissociate all endogenous oxytocin or arginine vasopressin from their binding sites. This is an important step because corpora lutea may contain significant amounts of oxytocin, which would interfere with the receptor assay, because of the relatively low specific activity of the available tritiated oxytocin (37 mCi/mmol). Membranes were suspended in calcium-free Hanks physiologic salt solution for storage at −70° to −80° C in a concentration of 1 mg/ml.

Binding assay. The assay measures the total number of binding sites in the particulate membrane fractions. The final protein concentration was 1:1.5 mg/ml in the assay buffer; the total volume of 220 μl/tube was increased to 400 μl/tube when >330 μg protein per tube was used. The assay buffer consisted of 0.05 mol/L Tris maleate, 5 mmol/L manganese chloride, 0.002% phenylmethylsulfonyl fluoride, and 0.1% bovine serum albumin pH 7.4. The labeled ligands were tritiated oxytocin (specific activity, 37 mCi/mmol) and tritiated arginine vasopressin (specific activity, 57.8 mCi/mmol) from New England Nuclear (Boston). The assay conditions were as previously described[18, 19]: incubation with shaking for 60 minutes at 22° C terminated by adding 5 ml ice-cold buffer and rapid filtration through Whatman CF/F glass fiber filters using a cell harvester (Brandel, Gaithersburg, Md.). The unlabeled ligands (purity: >99% by high-performance liquid chromatography verified by capillary electrophoresis) were obtained from Bachem (Torrance, Calif.). Other peptides were obtained from Peninsula, and other chemicals were from Sigma.

Saturation analysis. Analysis was done by use of six to eight different concentrations of labeled ligand ranging from 10^{-10} to 10^{-8} mol/L. Nonspecific binding was determined in the presence of 1.1×10^{-6} mol/L concentration of unlabeled ligand. All assays were done in duplicate.

Displacement and competition assays. These were performed with pooled bovine ovarian stromal membranes prepared from tissues obtained on days 17 and 21 of cycle. The following agonists were used: oxytocin; arginine vasopressin; isotocin, a highly selective oxytocin agonist; 1-deamino-8-D-arginine vasopressin (ddAVP), a highly selective antidiuretic agonist; and two selective oxytocin antagonists, 1 deamino[2-D-tyrosine (O-ethyl)-8-ornithine]oxytocin RWJ (provided by Dr. D. W. Hahn, Ortho Pharmaceutical Corp., Raritan, N.J.) and L365209 (provided by Dr. D. Pettibone, Merck, West Point, N.Y.). The binding specificity was tested by competition with human chorionic gonadotropin (94% purity, Steris Laboratories, Phoenix, Ariz.), and thyrotropin releasing hormone (Thypinone, Abbott Laboratories, North Chicago, Ill.).

Protein assays were performed according to the method of Lowry et al.[20] with bovine serum albumin as standard, and deoxyribonucleic acid (DNA) assays were performed according to the method of Burton[21] with calf thymus DNA as standard.

Data analysis. Analysis of the data was performed with a computerized nonlinear iterative curve-fitting program, LIGAND, which calculates the parameters K_a (association constant), K_d (dissociation constant), K_i (dissociation constant of inhibitor), and B_{max} (maximum binding) (Biosoft, Elsevier Science Publishers, New York).

Statistical analysis. The significance of the cyclic variations was assessed by analysis of variance followed by Dunnett's test using log-transformed data, and Student's *t* test was used to compare differences between two groups; values of $p < 0.05$ were considered significant. Values are given as mean ± SE unless otherwise indicated; *n* indicates the number of cows or number of pools from human ovaries, respectively.

Results

Preliminary results with bovine ovarian tissues indicated that the binding of tritiated oxytocin and tritiated arginine vasopressin was proportional to the protein concentration up to 1.5 mg/ml; at 2 mg/ml nonspecific interactions began to occur. Saturation analysis with 0.4 and 0.8 mg/ml of protein from the same bovine ovarian

stromal membrane preparation resulted in identical K_d and B_{max} values, with SEs <10%. Specific binding reached equilibrium at 22° C in approximately 45 minutes and was stable until 90 minutes; at +4° C the equilibrium was reached in 2 hours. At 37° C the results were erratic and specific binding had declined to low levels at 90 minutes. The presence of a divalent cation was essential for binding; calcium was ineffective and manganese was more effective than magnesium. Nonspecific binding constituted from 50% to 80% of total binding, depending on the concentration of labeled ligand and the concentration of protein in the saturation assay. Thyrotropin releasing hormone or human chorionic gonadotropin, in concentrations up to 10^{-6} mol/L, did not compete for the binding of labeled oxytocin. The precision of the assay was determined by measuring the binding to aliquots of a similar membrane fraction prepared from bovine endometrium obtained on the day of estrus. Scatchard plots on the binding data obtained with these quality control samples in 15 assays yielded similar parameters as the original analysis on the same tissue.[19]

High-affinity, saturable binding sites for tritiated oxytocin were found in all ovarian tissues, including the human pooled ovarian tissues, bovine ovarian stroma, corpora lutea, and follicles (Fig. 1). Tritiated arginine vasopressing binding was found in all tissues examined except the bovine corpora lutea, which showed no binding. The affinities were similar for both peptides and for all tissues with no significant variations between tissues or days of cycle (Table I). Unlabeled oxytocin displaced the labeled ligand with a K_d of 0.5×10^{-8} mol/L, and unlabeled arginine vasopressin competed for tritiated oxytocin with high affinity, K_i 0.8×10^{-9} mol/L. Labeled arginine vasopressin was displaced by arginine vasopressin with a K_d of 1.4×10^{-8} and oxytocin competed with tritiated arginine vasopressin with a K_i of 0.8×10^{-9}, indicating a high degree of cross-reactivity at the binding sites. The selective oxytocin agonist isotocin competed for tritiated oxytocin with high affinity, whereas the selective antidiuretic agonist ddAVP competed with much lower affinity. The two selective oxytocin antagonists RWJ22165 and L365209 also competed for tritiated oxytocin binding sites with similar affinity as in myometrial and endometrial membranes.[18] Fig. 2 shows the logit-log transformed competition curves for the tested agonists and antagonists.

The sizes of the follicles and corpora lutea in the ovaries of the experimental cows are shown in Table II together with plasma progesterone levels at the time of slaughter and the luteal oxytocin content, which was measured in corpora lutea of other cows from the same herd slaughtered on the same days of cycle. The measurements were performed by radioimmunoassay with

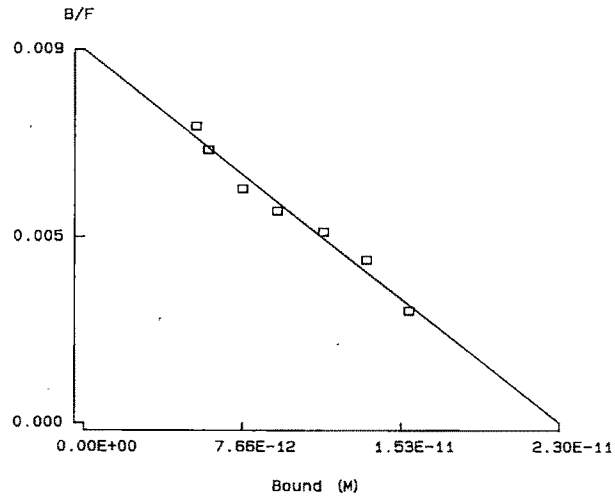


Fig. 1. Scatchard plot of data obtained in a saturation experiment with tritiated oxytocin binding to 400 μg of pooled bovine ovarian stromal membranes per tube. The ovaries were from days 17 and 21 of the estrous cycle.

Table I. Affinity constants of oxytocin and arginine vasopressin receptors in human ovarian tissue and bovine ovarian stroma, follicles, and corpora lutea obtained from saturation experiments

	Affinity constant (nmol/L)	
	Oxytocin	*Arginine vasopressin*
Human ovary, pooled	1.50 ± 0.4 (n = 5)	1.4 (n = 1)
Bovine ovarian stroma	1.53 ± 0.23 (n = 18)	2.10 ± 0.55 (n = 10)
Bovine follicles, pooled	0.92 ± 0.13 (n = 4)	0.45 (n = 1)
Bovine corpora lutea	2.55 ± 0.30 (n = 10)	No binding

Values are mean ± SE.

0.4 mol/L acetic acid extracts that were purified by absorption on Florisil columns with subsequent elution with 90% acetone water.

Human pooled ovarian tissues. The concentration of tritiated oxytocin binding sites per milligram of DNA rose progressively during the menstrual cycle from low levels in the proliferative phase (days 6 and 7), 71.7 ± 5 fmol/mg DNA, to 157 ± 60 mol/mg DNA in midcycle (days 14 to 18), and a peak of (652 mol/mg DNA) in the luteal phase (days 19 to 28). This value was more than three times the standard deviation above the mean for the proliferative phase and midcycle samples (Fig. 3). Expressed per milligram of protein, the variations during the cycle were similar; the concentration in the luteal phase was significantly higher than the combined mean for the proliferative phase and the midcycle val-

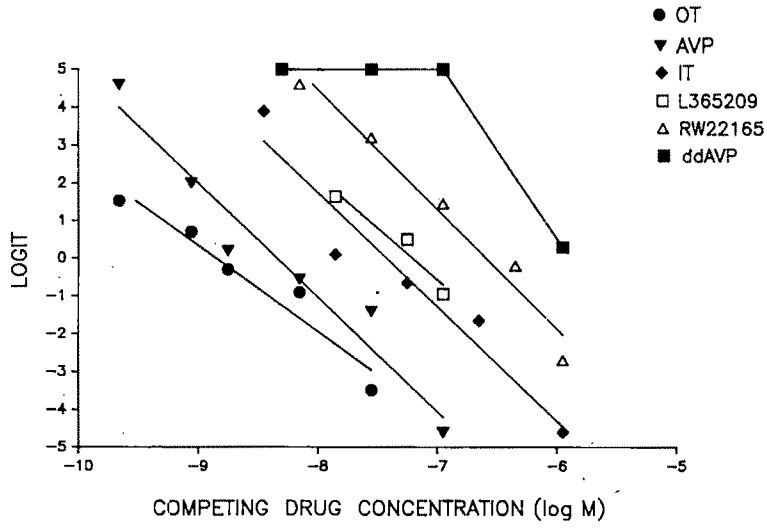


Fig. 2. Competition and displacement of tritiated oxytocin in membranes of pooled bovine ovarian stroma by various agonists *(closed symbols)* and antagonists *(open symbols)* plotted after logit-log transformation of the data. Specific binding in the absence of added unlabeled peptide was designated B_0. *IT*, Isotocin. L365209 and RWJ22165 are two oxytocin antagonists from Merck Co. and Ortho Corp., respectively.

Table II. Plasma progesterone levels, the size of the largest follicles, and weights of corpora lutea in the experimental cows, and luteal oxytocin content in cows of the same herd obtained on the same days of the cycle

Day	*Follicles* (mm)*	*Corpora lutea* (gm)*	*Plasma progesterone* (ng/ml)*	*Luteal oxytocin (pmol/gland)*
0	13.3 ± 3.1	1.3 ± 0.1	<0.2 ± 0	6
7	18.3 ± 2.4	3.7 ± 0.4	3.4 ± 0.2	1181
14	12.5 ± 3.6	4.6 ± 1.6	3.3 ± 0.4	2632
17	4.8 ± 1.3	3.6 ± 0.5	3.8 ± 1.9	227
19	—	3.0 ± 1.9	2.9 ± 1.2	195

Values are mean ± SE; n = 3 cows, except n = 2 for luteal oxytocin.

*Values are reproduced from Fuchs A-R, Behrens O, Helmer H, Liu C-H, Fields M, Barros C. Endocrinology 1990;127:629-39, courtesy of the Endocrine Society.

ues (results not shown). Tritiated arginine vasopressin binding was measured in only one pool because of a shortage of tissue. In that pool the femtomoles per milligram DNA concentration of the binding sites for tritiated arginine vasopressin was slightly higher than for tritiated oxytocin (Fig. 3).

Bovine ovarian stroma. The concentrations of oxytocin binding sites were lowest on day 14 (56 ± 22 fmol/mg DNA) and highest on day 21 (292 ± 64 fmol/mg DNA), but the variations were not significant by analysis of variance because of the considerable individual variations. When the values for the early and midluteal phase (days 7 and 14) and for the late luteal phase (days 17 to 21) were combined, the cyclic variations were statistically significant ($p < 0.05$); the combined late luteal phase values, 195 ± 41 fmol/mg DNA, were significantly higher than the combined early and midluteal phase values, 70 ± 15 fmol/mg DNA, ($p < 0.03$) (Fig. 4).

The concentration of arginine vasopressin binding sites was somewhat lower and showed a similar trend during the cycle as the oxytocin binding sites (54.1 ± 20 fmol/mg DNA on days 7 and 14 and 106 ± 48 fmol/mg DNA on days 17 to 21), but the differences were not statistically significant.

Expressed per milligram of protein, the cyclic variations were similar, and the values for tritiated oxytocin binding were significant by analysis of variance ($p < 0.05$, values not shown).

Bovine corpora lutea. Tritiated oxytocin binding sites were also found in the corpora lutea. The concentrations were very low or nondetectable in early and midluteal phase (days 7 and 14, 42 ± 35 fmol/mg DNA), but in late luteal phase (on days 17, 19, and 21), the concentrations of binding sites were significantly increased; the mean value for these days was 616 ± 93 fmol/mg DNA ($p < 0.005$) (Fig. 5). Maximal values were observed on day 19 (3740 ± 209 fmol/mg DNA). The old corpora lutea present on day 0 had concentrations varying from 263 to 602 fmol/mg DNA.

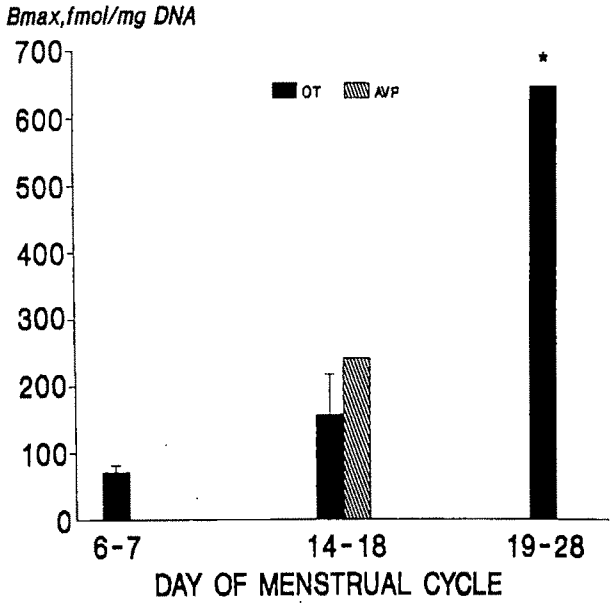


Fig. 3. Concentrations of specific binding sites for tritiated oxytocin and tritiated arginine vasopressin in human ovarian membrane preparations expressed in femtomoles per milligram of DNA. Tissues from two to three premenopausal patients were combined according to the phase of menstrual cycle to obtain sufficient tissue for saturation analysis. Two pools were analyzed for oxytocin binding on days 6 and 7 and 14 to 18, and one pool for oxytocin binding was analyzed on days 19 to 28 and for arginine vasopressin binding on days 14 to 18. *Asterisk* denotes significant difference from the combined means of the two other groups analyzed for oxytocin binding, $p < 0.01$.

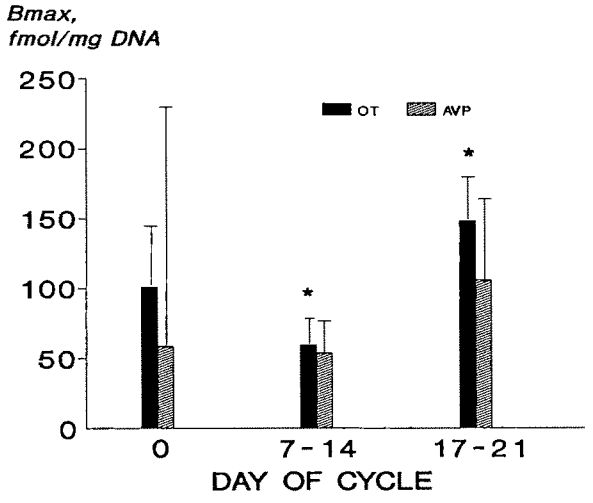


Fig. 4. Concentrations of specific binding sites for tritiated oxytocin and tritiated arginine vasopressin in bovine ovarian membrane preparations expressed in femtomoles per milligram of DNA. Large follicles and corpora lutea had been removed from the tissues. Values are log-normal mean ± SE. Results obtained for days 7 and 14 and for days 17 to 21 were combined. *Asterisks* denote groups that differed significantly.

No specific binding of tritiated arginine vasopressin was found on any day.

Bovine follicles. The largest follicle was dissected from each ovary and analyzed for tritiated oxytocin and tritiated arginine vasopressin binding sites. To obtain sufficient material for saturation analysis, all follicles from the same day of cycle were pooled. High-affinity, saturable binding sites for oxytocin were present on each day examined. The concentrations per milligram of DNA were higher than in the stroma. The concentration was highest on day 0 (475 fmol/mg DNA) and lowest on day 7 (76.2 fmol/mg DNA) and then increased progressively during the luteal phase to 308 fmol/mg DNA on day 17 (Fig. 6). Tritiated arginine vasopressin was bound with about equal affinity as tritiated oxytocin on day 0 (the only day examined for arginine vasopressin binding) but the concentration was about 50% lower than tritiated oxytocin binding, 292.5 fmol/mg DNA.

Comment

To our knowledge, this is the first demonstration of specific binding sites for the neurohypophysial peptides, oxytocin and arginine vasopressin, in bovine and human ovarian tissues. Corpora lutea of pregnant ewes were recently shown to bind tritiated oxytocin, whereas no binding was found in the corpora lutea of nonpregnant ewes.[22] In the nonpregnant cow, binding sites for tritiated oxytocin were present in the corpora lutea as well as in ovarian stroma and large follicles. The competition studies with selected agonists and antagonists with well-defined pharmacologic actions were compatible with the notion that the oxytocin binding sites represent biologic receptors. Whether the tritiated arginine vasopressin binding sites represent separate receptors cannot be stated on the basis of these results. The ovarian oxytocin binding sites resemble those in the uterus in their affinity to various analogs and in the cross-reactivity with arginine vasopressin.[18]

The results suggest that the two neuropeptides play a role in the regulation of ovarian function. Such a role has been postulated for oxytocin in sheep and cows on the basis of several physiologic observations, including the lengthening of estrus cycles in animals immunized against oxytocin,[23-25] and shortening of cycles in animals given injections of oxytocin at appropriate stages of the cycles.[11, 12] The effects of oxytocin on luteal function are best known in the ewe in which the action of oxytocin is thought to be indirect and mediated by $PGF_{2\alpha}$ released from the uterus under the influence of oxytocin.[3, 14, 15] In the cow, the influence of oxytocin on luteal function is still somewhat controversial. Lengthening or shortening of the cycle length has been reported, depending on the day of cycle oxytocin is administered.[11, 26] Oxytocin receptors are found in the cow endometrium in late luteal phase and around estrus,[19] on days when oxytocin has the ability to stimulate $PGF_{2\alpha}$

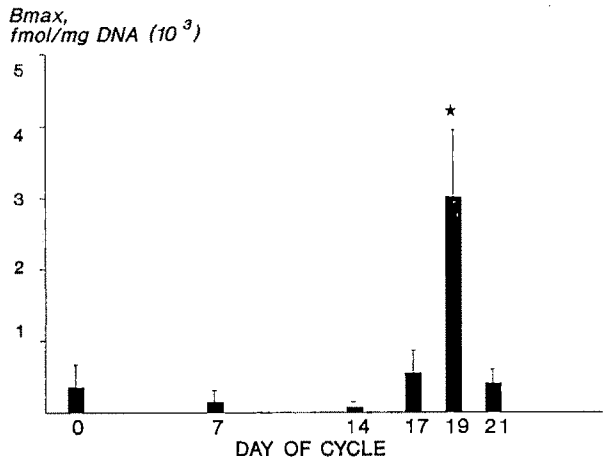


Fig. 5. Specific binding of tritiated oxytocin to membrane preparations from bovine corpora lutea expressed in femtomoles per milligram of DNA. Note that ordinate is in picomoles per milligram DNA (fmol × 10^3). *Asterisk* indicates significant differences from all other groups, $p < 0.005$ (analysis of variance). No specific tritiated arginine vasopressin binding was observed on any day of cycle.

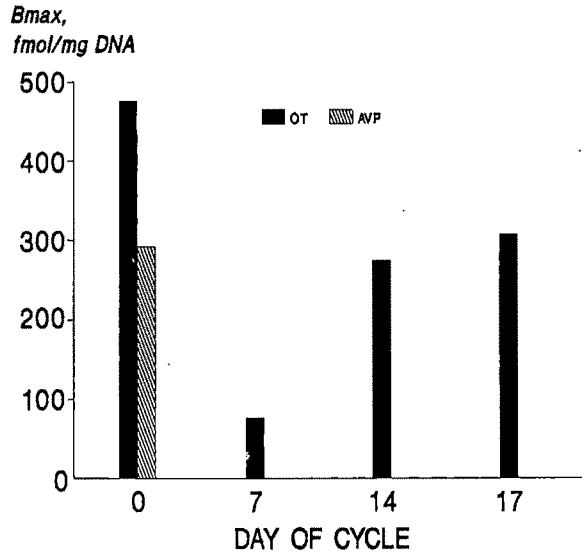


Fig. 6. Specific binding of tritiated oxytocin and tritiated arginine vasopressin to membrane preparation from large bovine follicles expressed in femtomoles per milligram of DNA. All follicles from cows killed on the same day of cycle were pooled to obtain sufficient material for saturation analysis. Tritiated arginine vasopressin binding was measured in only one pool, consisting of follicles from days 0 and 21 combined.

release from the endometrium in vitro[27] and increase uterine vein $PGF_{2\alpha}$ levels in vivo.[28] At least part of the oxytocin effect on luteal function in the bovine may therefore be mediated by uterine $PGF_{2\alpha}$ release as it is in sheep. However, our results suggest that oxytocin may also have direct effects on the ovary in general and corpus luteum in particular, since the rise in luteal oxytocin receptor concentrations was coincident with the beginning of the decline in plasma progesterone, reported earlier[19] and shown in Table II. The failure to show significant effects of oxytocin on luteal progesterone production in vitro may be a result of the fact that most investigators have used luteal tissue obtained on days 7 to 14, when oxytocin binding sites are at their nadir.

Bovine stromal and human ovarian oxytocin receptor concentrations also were increased when luteal regression began. The ability of oxytocin to effect the release of $PGF_{2\alpha}$ from the ovarian stroma has been demonstrated in rat ovaries.[29] Our findings therefore are compatible with a paracrine role for ovarian oxytocin in luteolysis in the bovine and possibly in the human. The synthesis of oxytocin in bovine,[8,9] ovine,[3] and porcine[4] ovaries and corpora lutea is firmly established, whereas the synthesis of oxytocin in primate ovaries is still controversial.[3,6] However, the recent demonstration of oxytocin production by human granulosa cells in culture is a strong indication of the ability of human ovaries to synthesize oxytocin de novo.[10] It is significant that arginine vasopressin binding sites also were present in human ovaries, since primate ovaries were recently found to secrete significantly more arginine vasopressin than oxytocin in the late luteal phase.[30]

The stromal oxytocin and arginine vasopressin receptors may also mediate the contractile effects of the neuropeptides on the ovary.[31,32] Myoepithelial cells have been identified in perifollicular tissue[33] and contractile proteins were identified in thecal cells [34] Although the localization of oxytocin and arginine vasopressin receptors within the ovary remains to be established, the smooth muscles like perifollicular cells located in the theca are likely sites for such receptors. It is also possible that arginine vasopressin receptors are located in ovarian vascular tissue and are involved in the regulation of ovarian blood flow.[35] It is not yet known whether the ovarian arginine vasopressin receptors are of the VP1 type present in vascular tissue or the uterine type present in endometrium and myometrium.[18,19]

The finding of oxytocin binding sites in follicles indicates that oxytocin may have actions other than the luteolytic and contractile effects in ovarian function. Oxytocin has been implicated in the process of ovulation in the rat,[28] acting perhaps on the outermost theca cells expressing muscle characteristics.[34]

In conclusion, human ovarian tissue and bovine ovine stroma, large follicles, and corpora lutea possess specific tritiated oxytocin binding sites. With the exception of bovine corpora lutea, tritiated arginine vasopressin binding sites were also found in ovarian tissues. In humans, the concentrations of tritiated arginine vasopressin binding sites were similar as tritiated oxytocin; in the bovine they were somewhat lower. The binding sites showed considerable cross-reactivity be-

tween the two hormones. The competiton for the specific binding of tritiated oxytocin by the two oxytocin antagonists suggests that the binding sites represent receptors. Their concentrations varied during the estrous cycle and were low in human proliferative phase and in early human and bovine luteal phase with a significant rise in late luteal phase in both species. These variations are compatible with the hypothesis that oxytocin has a paracrine role in the regulation of luteal function in both species. The binding sites in the stromal tissue and follicles may also mediate contractile responses to the two neuropeptides. The presence of high concentrations of binding sites in periovulatory follicles suggests that oxytocin and perhaps arginine vasopressin may play a physiologic role in follicular rupture.

REFERENCES

1. Fields PA, Fields MJ, Eldridge RK, Fuchs A-R. Human placental and bovine corpora luteal oxytocin. Endocrinology 1983;112:1544-6.
2. Wathes DC, Swann RW, Birkett SD, Porter DG, Pickering BT. Characterization of oxytocin, vasopressin and neurophysin from the ovine corpus luteum. Endocrinology 1983;113:693-8.
3. Flint APF, Auletta RJ. Detection and identification of luteal peptides: limitations of current techniques exemplified by the study of oxytocin. Semin Reprod Endocrinol 1990;8:107-14.
4. Einspamer R, Pitzel L, Wuttke W, et al. Demonstration of messenger RNAs for oxytocin and prolactin in porcine granulosa and luteal cells. FEBS Lett 1986;204:37-40.
5. Fuller PJ, Clements JA, Tregear GW, Nikolaidis T. Vasopressin-neurophysin II gene expression in the ovary: studies in Sprague Dawley, Long Evans and Brattleboro rats. J Endocrinol 1985;105:317-21.
6. Dawood MY, Khan Dawood FS. Human ovarian oxytocin: its source and relationship to steroid hormones. Am J Obstet Gynecol 1986;154:756-63.
7. Khan Dawood FS, Marut EL, Dawood MY. Oxytocin in the corpus luteum of the cynomolgus monkey *(Macaca fascicularis)*. Endocrinology 1984;115:570-4.
8. Fehr S, Ivell R, Koll R, Schams D, Fields M, Richter D. Expression of the oxytocin gene in the large cells of the bovine corpus luteum. FEBS [Letter] 1987;210:45-50.
9. Jungclas B, Luck MR. Granulosa theca interaction and oxytocin secretion. J Endocrinol 1986;109:R1-R4.
10. Plevrakis I, Clamagirand C, Pontonnier G. Oxytocin biosynthesis in serum-free cultures of human granulosa cells. J Endocrinol 1990;124:R5-R8.
11. Armstrong DT, Hansel W. Alteration of the bovine estrous cycle with oxytocin. J Dairy Sci 1959;42:533-42.
12. Cooke RG, Knifton A. Oxytocin-induced oestrus in the goat. Theriogenology 1981;16:95-7.
13. Auletta FJ, Paradis DK, Wesley M, Duby RT. Oxytocin is luteolytic in the rhesus monkey. J Reprod Fertil 1984; 72:401-6.
14. Sharma SC, Fitzpatrick RJ. Effect of oestradiol-17β and oxytocin treatment on $PGF_{2\alpha}$ release in anestrous ewe. Prostaglandins 1974;6:997-106.
15. Roberts JS, McCracken JA, Gavagan JE, Soloff MS. Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vitro: correlation with estrous cycle and oxytocin receptor binding. Endocrinology 1976;99: 1107-11.
16. Auletta FJ, Kamps DL, Pories S, Bisset J, Gibson M. An intra-corpus luteum site for the luteolytic action of prostaglandin $F_{2\alpha}$ in the rhesus monkey. Prostaglandins 1984; 27:285-98.
17. Fuchs A-R. Oxytocin and ovarian function. J Reprod Fertil 1988; suppl 36:39-47.
18. Ivanisevic M, Behrens O, Helmer H, Fuchs A-R. Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists. Am J Obstet Gynecol 1989;161: 1639-43.
19. Fuchs A-R, Behrens O, Helmer H, Liu C-H, Fields M, Barros C. Oxytocin and vasopressin receptors in the bovine endometrium and myometrium during the estrous cycle and early pregnancy. Endocrinology 1990;127:629-39.
20. Lowry OH, Roselbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
21. Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956;62:315-23.
22. Sernia C, Gemmel RT, Thomas WG. Oxytocin receptors in ovine corpus luteum. J Endocrinol 1989;121:117-23.
23. Sheldrick EL, Mitchell MD, Flint APF. Delayed luteal regression in ewes immunized against oxytocin. J Reprod Fertil 1980;59:37-42.
24. Wathes DC, Ayad VJ, McGoff SA, Morgan KL. Effect of active immunization against OT on gonadotropin secretion and the establishment of pregnancy in the ewe. J Reprod Fertil 1989;86:653-64.
25. Schams D, Prokopp S, Barth D. The effect of active and passive immunization against oxytocin on ovarian cyclicity in ewes. Acta Endocrinol 1983;103:337-44.
26. Gilbert CL, Lamming GE, Parkinson TJ, Flint APF. Oxytocin infusion from day 10 after oestrus extends the luteal phase in non-pregnant cattle. J Reprod Fertil 1989;86: 203-10.
27. LaFranca M, Goff AK. Effect of pregnancy on oxytocin-induced release of prostaglandin $F_{2\alpha}$ in heifers. Biol Reprod 1985;33:1113-19.
28. Oyedipe EO, Gustafsson B, Kindahl H. Blood levels of progesterone and 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ during the estrous cycle. Theriogenology 1984;22: 329-39.
29. Viggiano M, Franchi A, Zicari JL, Gimeno MA. The involvement of oxytocin in ovulation and in the output of cyclo-oxygenase and 5-lipoxygenase products from isolated rat ovaries. Prostaglandins 1989;37:367-78.
30. McCracken JA, Hoberg LA, Longcope C, Robinson AG. Vasopressin and oxytocin in ovarian venous blood and tissues during the luteal phase of the rhesus menstrual cycle. Biol Reprod 1988;38[suppl 1]:192.
31. Coutinho EM, Maia H, Maia H Jr. Ovarian contractility. In: Coutinho EM, Fuchs F, eds. Physiology and genetics of reproduction. New York: Plenum, 1974:127-37. (Hollander A, ed. Basic life science; vol 4, part B).
32. Virutamasen P, Wright KH, Wallach EE. Monkey ovarian contractility—its relationship to ovulation. Fertil Steril 1973;24:763-71.
33. Burden HW. Ultrastructural observations on ovarian perifollicular smooth muscle in the cat, guinea pig and rabbit. Am J Anat 1972;133:125-42.
34. Self Da, Schroeder PC, Gown AM. Hamster thecal cells express muscle characteristics. Biol Reprod 1988;39:119-30.
35. Varga B, Horvath E, Zsolnai B. Effects of oxytocin and vasopressin on the ovarian blood flow, progesterone and oestradiol-17beta secretion in oestrous rats. Acta Endocrinol [Copenh] 1985;110:271-5.

Correlation of the testicular determinant factor sequence zinc finger Y with varying gonadal phenotypes in a series of 13 subjects with gonadal dysgenesis due to Y aneuploidy

Sandra P.T. Tho, MD,[a] Ali Behzadian, PhD,[a, b] J. Rogers Byrd, PhD,[c] and Paul G. McDonough, MD[a]

Augusta, Georgia

Deoxyribonucleic acid samples from a series of 13 subjects with 45,X/46,X,altered Y, and varying gonadal phenotypes (streak-streak, $n = 9$; streak-testis, $n = 2$; testis-testis, $n = 2$) were analyzed for the presence of the candidate testicular determinant factor sequence zinc finger Y.[1] The Y-specific probes Y97 mapped to Y centromere, pDP105 A,B mapped to Yp and distal Yq11, respectively, hybridized with the deoxyribonucleic acid from all the 13 study subjects. The same deoxyribonucleic acid samples were analyzed for the presence of the zinc finger Y sequence. Eleven of the 13 subjects were positive for the zinc finger Y sequence. Four zinc finger Y–positive subjects had unilateral ($n = 2$) or bilateral ($n = 2$) testicular differentiation. Among the nine subjects with bilateral streak gonads, seven showed the presence of this sequence. The lack of testicular differentiation in the presence of quantitatively normal or almost normal zinc finger Y bands could not be explained by mosaicism alone. Mutations not detectable by analysis with the method of Southern with pDP1007, may occur in the testicular determinant factor gene vitiating testicular development. (Am J Obstet Gynecol 1990;163:1968-75.)

Key words: Testicular determinant factor, zinc finger Y, zinc finger X, mixed gonadal dysgenesis, Turner syndrome

Chromosomal constitutions derived from Y aneuploidy cover a wide range of 45,X/46,XY mosaicisms with varieties of Y morphology including normal Y, nonfluorescent minutes, and large fluorescent dicentric fragments. The resultant phenotypes vary from classic Turner female with bilateral rudimentary streaks to normal male phenotype with bilateral atrophic scrotal testes through degrees of sexual ambiguity with unilateral abdominal streak and contralateral abdominal or scrotal testis.[2, 3] In rare subjects with typical Turner phenotypes, unilateral streak and contralateral testis, the expected 45,X cell line may have been lost with time. These unique subjects remain nonmosaic 46,XY on the basis of chromosomal analysis of multiple tissues.[4, 5] Conversely, the 46,XYcell line may disappear and the subject remains nonmosaic 45,X, yet carrying a unilateral streak gonad and a contralateral testis.[6] There does not seem to be a consistent karyotypical gonadotypical correlation because the same degrees of mosaicism and similar structural alterations of the Y chromosomal fragments may be associated with either a typical Turner female harboring bilateral streaks or a normal male with bilateral scrotal testes or a sexually ambiguous individual with classic mixed gonadal dysgenesis. Recently, by chromosome walking, Page and coworkers[1] isolated a 140 kilobase (kb) region of Yp, which initially was thought to be the sole testicular determinant factor gene. A deoxyribonucleic acid (DNA) probe, pDP1007, isolated from an exon of the coding region of this gene yields a Y-specific band mapped to distal Yp and a smaller molecular weight X-specific band mapped to Xp22.1-p22.2. Sequence analysis indicated that the Y-specific sequence encodes a DNA binding protein with zinc finger domains. This sequence has been called zinc finger Y. The homologous sequence on X has been referred to as zinc finger X.[7] Furthermore, zinc finger Y has proved to be conserved in most mammals. Despite much controversy,[8-11] the zinc finger Y sequence by its conserved nature and its regulatory function appears to play an important role in testicular differentiation.

The frequency of Y chromosomal rearrangements and the lack of karyotype-gonadotype correlation have prompted us to assess the presence or absence of the zinc finger Y sequence in these structurally compromised Y-derived fragments. Comparison of molecular findings with gonadal phenotypes in a series of 13 subjects with 45,X/46,X,altered Y is the primary objective

From the Departments of Obstetrics and Gynecology,[a] Anatomy,[b] and Endocrinology and Physiology,[c] Medical College of Georgia.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Sandra P. T. Tho, MD, Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA 30912-3360.
6/6/24752

of this report. The study also provides a molecular deletion mapping of a wide variety of structurally rearranged Y chromosomes in gonadal dysgenesis due to Y aneuploidy.

Material and methods

Thirteen of the 22 subjects with gonadal dysgenesis and 45,X/46,X,mar karyotype were ascertained to carry Y DNA with two Y-specific probes: Y97 mapped to Y centromere, pDP105 mapped to Yp and Yq11. The remaining nine subjects who were negative for these two probes were presumed to harbor X-derived markers and were not included in the study. The Y-specific probe 4B-2 mapped to Yq11 was used for delineation of the break points of these Y fragments. The 13 subjects were also analyzed for the candidate testicular determinant factor probe pDP1007 and attempts were made to compare testicular differentiation with the presence of the zinc finger Y sequence.

DNA probes. Fig. 1 illustrates the schematic map of the Y chromosome with the seven deletion intervals of Vergnaud et al.[12] and representation of the four Y DNA probes used in this study. The cosmid Y97 (DYZ3) isolated from a cosmid Y library contains one copy of the 5.5 kb *Eco*RI fragment of the Y centromere–specific alphoid DNA. Human genomic DNA restricted with *Eco*RI and hybridized to the Y97 probe shows a 5.5 kb Y-specific band.[13] The probe pDP105 (DYZ4) isolated by Page is a 5.5 kb *Hin*dIII genomic fragment inserted into the *Hin*dIII site of pUC13. This probe hybridizes with *Taq* I digested human DNA, yielding a 2.5 kb Y-specific band (pDP105/A), marker for interval 3 on Yp, and a 5.2 kb Y-specific band (pDP105/B), marker for interval 6 on Yq11.[14] The probe 4B-2 (DYS15) is a recombinant phage construct containing a 3.3 kb single copy *Eco*RI fragment that maps to Yq11. This 3.3 kb fragment resulting from *Eco*RI digest of the recombinant phage was subcloned into the *Eco*RI site of plasmid pBR322.[15] Human DNA restricted with *Eco*RI and hybridized to the 4B-2 probe yields the Y-specific 3.3 kb band, marker for interval 5 on Yq11. The probe pDP1007 (zinc finger Y, zinc finger X) is a 1.3 kb *Hin*dIII genomic fragment cloned into the *Hin*dIII site of pUC13. It derives from interval 1A2 of the human Y chromosome. Genomic human DNA digested with *Eco*RI and hybridized with pDP1007 exhibits a Y-specific 3.5 kb band and an X-specific 1.8 kb band, mapped to Xp22.1-p22.2.[1] The cosmid Y97 and the plasmids pDP105, 4B-2, and pDP1007 were propagated, amplified, and isolated by conventional methods.[16] The corresponding inserts were released from their vectors by restriction digest with the appropriate enzymes and purified through electrophoresis, phenol extraction, and ethanol precipitation. Before hybridization, the probe pDP1007 was radiolabeled by nick translation[17] and the other three probes by oligonucleotide random priming[18] to specific activity of 1 to 5×10^8 cpm/μg DNA.

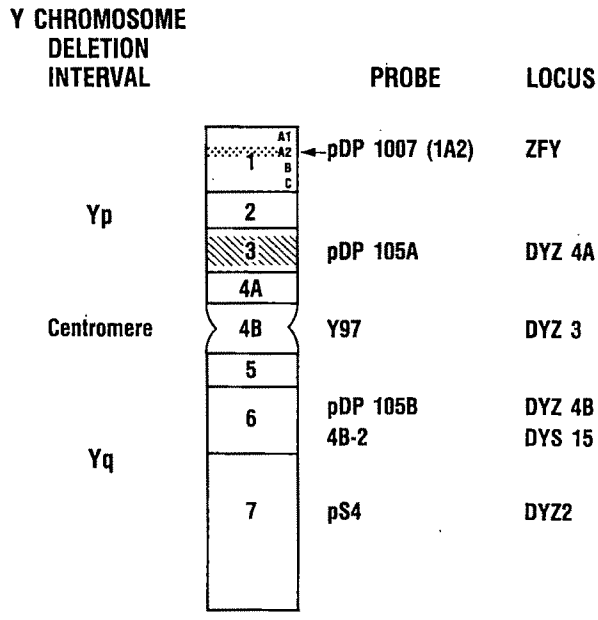


Fig. 1. Schematic map of Y chromosome with representation of seven deletion intervals of Vergnaud[12] and four Y-specific probes Y97, pDP105, 4B-2, and pDP1007. *DYZ2:* D = DNA, Y = Y chromosome, Z = repetitive copy DNA, 2,3,4 indicate published order of specific cloned DNA sequence; *DYS15:* D = DNA, Y = Y chromosome, S = single copy, 15 indicates published order of cloned DNA sequence; *ZFY:* ZF = Zinc finger protein encoding sequence, Y = on Y chromosome.

Genomic DNA analysis. Human genomic DNA was extracted from peripheral leukocytes from normal control 46,XY men, 46,XX women, and from the 13 study subjects according to standard techniques. Aliquots of DNA were digested with the enzymes *Eco*RI for probes Y97, 4B-2, and pDP1007 and *Taq* I for the probe pDP105, and electrophoresed in 0.8% agarose gels. DNA was transferred to Gene Screen Plus filters (New England Nucleic Research Products, Boston) by the technique of Southern[19] and baked for 2 hours at 80° C. The blots were hybridized for 48 hours at 42° C with formamide to the ^{32}P-labeled probe pDP1007 and at 65° C to ^{32}P-labeled probes Y97, pDP105, and 4B-2. The blot hybridized with pDP1007 was washed at 55° C with decreasing concentrations of sodium chloride-sodium citrate (2 × sodium chloride-sodium citrate to 0.2 × sodium chloride-sodium citrate) and 1% sodium dodecyl sulfate. The blots hybridized with the other three probes were washed at high stringency conditions at 65° C with decreasing concentrations of sodium chloride-sodium citrate (2 × sodium chloride-sodium citrate to 0.2 × sodium chloride-sodium ci-

Table I. Tabulated clinical, cytogenetic, and molecular data on 13 study subjects

Case	Age at first visit or consultation	Phenotype	Karyotype		Morphology of altered Y	Y97 EcoRI	pDP105 Taq I	4B-2 EcoRI	pDP1007 EcoRI
			% 46X, altered Y	Cells counted					
1	15	Female with BSG	6	50	marker	+	A+,B+	+	+
2	1 week	Female with Rt. intraabdominal SG Lt. scrotal testis	50	50	marker	+	A+,B+	−	+
3	15	Female with Rt. intraabdominal SG Lt. intraabdominal testis	84 idicYq	50	idic(Y)qter→p11::p11→qter	+	A+,B+	+	+
4	16	Female with BSG	14	50	marker	+	A+,B+	+	+
5	14	Female with BSG	66	50	marker	+	A+,B+	+	+
6	4 months	Female with bilateral intraabdominal testes (Lt. sliding hernia)	11	100	marker	+	A+,B+	−	+
7*	18	Female with BSG	68	50	marker	+	A+,B+	−	−
8*	27	Female with BSG	18	50	marker	+	A+,B+	−	−
9*	13	Female with BSG	10	50	ring(?)	+	A+,B+	−	+
10	5	Female with BSG	74	50	marker	+	A+,B+	+	+
11*	22	Female with BSG	92	50	marker	+	A+,B+	−	+
12	28	Female with BSG	16	50	idic(Y)pter→q12::q12→pter	+	A+,B+	+	+
13	7	Male bilateral scrotal testes	94	50	marker	+	A+,B+	−	+

BSG, Bilateral streak gonads; *Rt.SG,* right streak gonad; *Lt.SG,* left streak gonad; *marker,* ill-defined chromosomal fragment; *ring(?),* ring of unknown origin.

*Patients with dysgenetic tumors; patients 7, 9, and 11, gonadoblastoma; patient 8, dysgerminoma.

trate) and 1% sodium dodecyl sulfate. Autoradiographs were obtained by exposing the membranes with intensifying screens at −80° C from 1 to 3 days to Kodak XAR-5 film (Eastman Kodak Company, Rochester, N. Y.).

Specific experiments

Experiment I. Dilutional gel for probe pDP105. Decreasing amounts of normal 46,XY male DNA (5 to 0.025 μg) were mixed with normal 46,XX female DNA, digested with *Taq* I enzyme, and hybridized to the ^{32}P-labeled pDP105 probe at 65° C for 48 hours (5 μg/lane).

Experiment II. Dilutional gel for probe pDP1007. Decreasing amounts of normal 46,XY male DNA (5 to 0.025 μg) were mixed with normal 46,XX female DNA, digested with *Eco*RI enzyme, and hybridized to the ^{32}P-labeled pDP1007 probe at 42° C with formamide for 48 hours (5 μg/lane).

*Experiment III. Eco*RI *digest and pDP1007 probing of DNA from the 13 study subjects.* The clinical, cytogenetic, and molecular data are tabulated in Table I.

Clinical data. The clinical phenotype of the 13 subjects ranges from phenotypic male with bilateral scrotal testes (No. 13) to classic Turner phenotype with bilateral streak gonads (n = 9) and sexually ambiguous individuals (No. 2, streak-scrotal testis; No. 3, streak-abdominal testis; No. 6, bilateral abdominal testes). The three latter subjects, each with a vagina and a uterus on vaginoscopy and urethroscopy, have been reared as females and underwent clitoral resection and bilateral gonadectomy. Gonadal tumor occurred in four of the 13 subjects. In 3 subjects, a gonadoblastoma developed in one of the streaks (No. 7, No. 9, and No. 11) and the remaining one had a unilateral dysgerminoma (No. 8).

Cytogenetic studies. Chromosomal analysis was per-

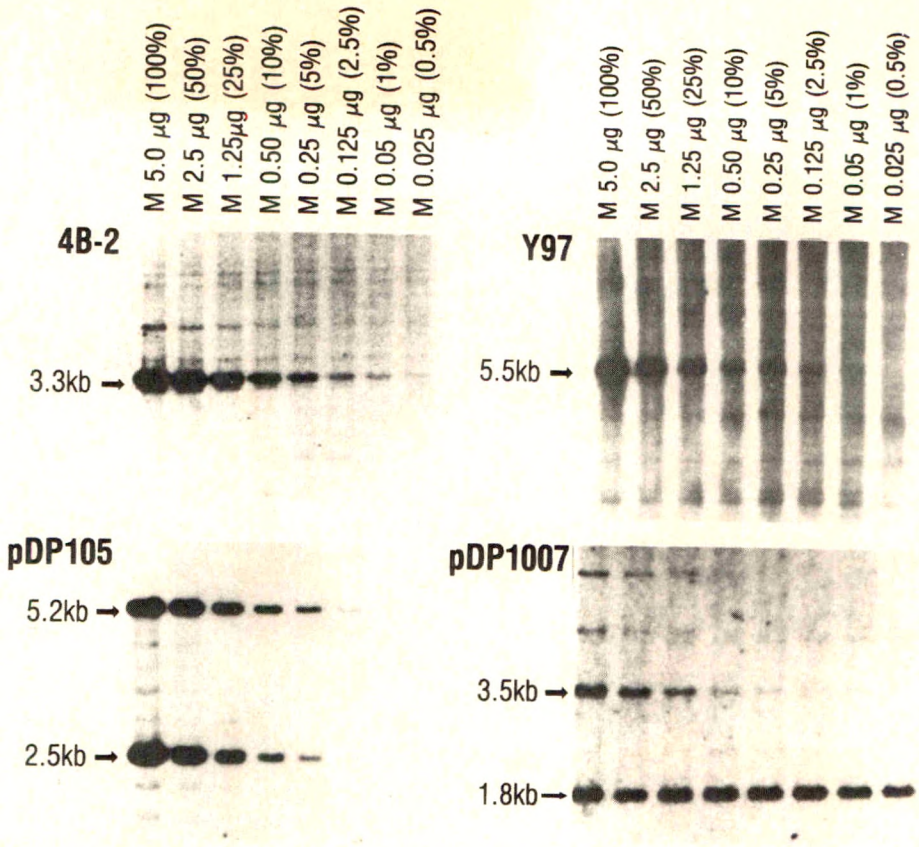


Fig. 2. Composite of four dilutional gels for probes 4B-2, Y97, pDP105, and pDP1007. All four dilution experiments were conducted in same fashion. Each lane contained 5 μg of DNA digested by appropriate enzymes and the blots of these four dilutional gels were hybridized separately to the four probes. *First lane on left* contained only male DNA (5.0 μg, or 100% male DNA). *Next lane* contained 2.5 μg male DNA (2.5 μg or 50% male DNA). Proportion of male DNA *(M)* gradually decreases to 0.5%. Y-specific 3.3 kb band with 4B-2 is seen at levels as low as 0.025 μg or 0.5% male DNA; 5.5 kb band with Y97 at 0.050 μg or 1% male DNA; 5.2 kb and 2.5 kb bands with pDP105 at 0.125 μg or 2.5% male DNA and 3.5 kb band with zinc finger Y at 0.250 μg or 5% male DNA.

formed on peripheral leukocytes in all subjects. Because cytogenetic studies on gonadal and nongonadal tissues are not available in some patients and because there are no good correlations between the numeric prevalence of the 46,XY cell line and testis formation,[2] the current investigation is limited to peripheral blood cells. A minimum of 50 cells was counted and five cells were karyotyped in each study sample. G-, Q-, and C-banding was carried out in all 13 cases. The altered sex chromosome was cytogenetically identified as Y-derived only in patient No. 3 who carries a large fluorescent isodicentric Y with undetermined break point on Yp. In patient No. 12, the sex chromosome was presumed to be Y-derived on the basis of the presence of a bright fluorescent band in the middle of an enlarged chromosome long arm. All the remaining cytologically altered chromosomal fragments were unidentifiable by conventional cytogenetic techniques.

DNA analysis of the study subjects. Aliquots of 10 μg of DNA from the 13 subjects with gonadal dysgenesis due to Y aneuploidy were analyzed with the four probes Y97, pDP105, 4B-2, and pDP1007.

Results

Experiment I. In dilution studies, the probe pDP105, a marker of interval 3 on Yp and of interval 6 on Yq11, may detect 125 ng of male DNA in mixture with 4.875 μg of female DNA (2.5%) (Fig. 2). We previously showed that the Y centromere–specific probe Y97 may detect 50 ng of male DNA in mixture with 4.950 μg female DNA (1%) and that the probe 4B-2, a marker for Yq11, may detect 25 ng of male DNA in mixture with 4.975 μg of female DNA (0.5%) (Fig. 2).

Experiment II. In dilution studies the probe pDP1007 mapped to distal Yp may detect 250 ng of male DNA in mixture with 4.750 μg of female DNA (5%) (Fig. 2).

Experiment III. (1) Southern analysis clearly showed the Y centromeric 5.5 kb *Eco*RI band with the Y centromere–specific probe Y97 in all 13 subjects. The

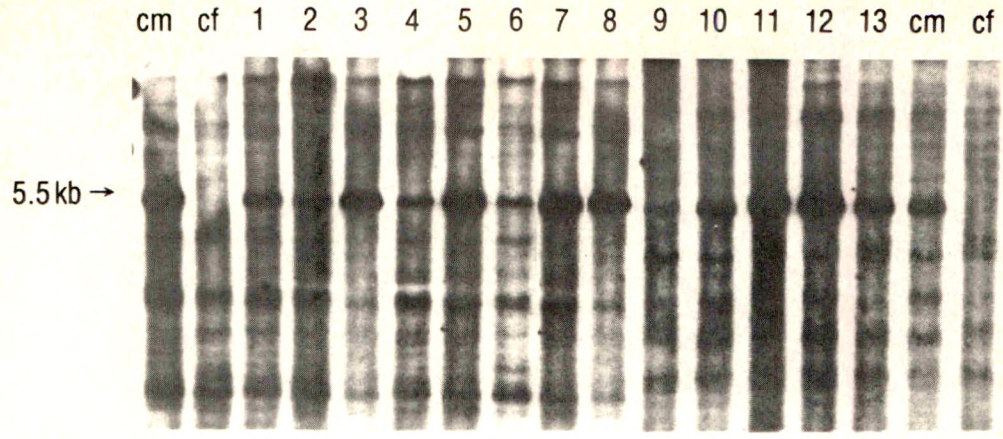


Fig. 3. Autoradiograph of *Eco*RI digest and Y97 probing of 13 study subjects (Nos. 1 to13). Centromeric Y-specific 5.5 kb *Eco*RI band is evident in control males *(cm)* and in all 13 subjects. It is absent in control females *(cf)*.

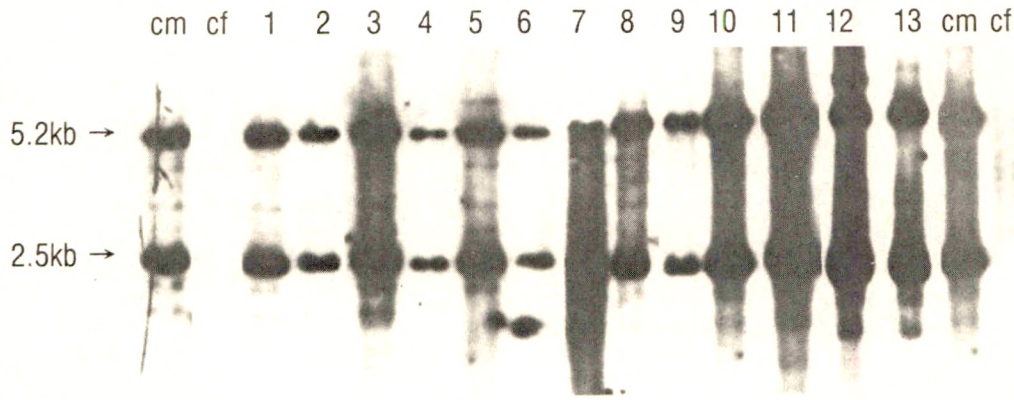


Fig. 4. Autoradiograph of *Taq* I digest and pDP105A/B probing of 13 study subjects. Y-specific 2.5 kb *Taq* I band, marker of interval 3 of Yp (pDP105/A), and 5.2 kb *Taq* I band, marker of interval 6 of Yq11 (pDP105/B), are seen in control males *(cm)* and in all 13 subjects. They are absent in control females *(cf)*.

intensity of the bands is in gross agreement with the importance of the mosaic Y cell lines (Fig. 3); (2) the Y-specific 2.5 kb *Taq* I hybridization band with the pDP105/A sequence, marker of Yp at interval 3, and the 5.2 kb *Taq* I hybridization band with the pDP105/B sequence, marker of Yq11 at interval 6, are also evident in all 13 subjects. The intensity of these two bands is mostly consistent with the percentage of the mosaic Y-bearing cell lines (Fig. 4); (3) the Y-specific 3.3 kb *Eco*RI hybridization band with the 4B-2 probe, marker of Yq11, is present only in six of the 13 study subjects. The break point in four (No. 1, No. 4, No. 5, and No. 10) of these six subjects is probably at the Yq11-Yq12 junction. In the fifth subject (No. 3), Yq is intact and the break point would be in terminal Yp. Fusion of the two Y chromosomes at their break points results in the formation of a isodicentric Y. In the sixth subject (No. 12), the break point is within Yq12 and fusion of the two Y chromosomes at their break points results in an unusual isodicentric Y chromosome with a fluorescent band in the middle of an enlarged derivative Yq. In the seven 4B-2–negative subjects the break point would be at the junction of the pDP105/B and the 4B-2 sequences within distal Yq11 (Fig. 5); (4) the Y-specific 3.5 kb *Eco*RI hybridization band with the pDP1007 probe is present in 11 of the 13 study subjects. The intensity of the band is compatible with the proportion of Y cells in nine of the 11 zinc finger Y–positive subjects. In the tenth subject (No. 1), the band is darker than expected and in the eleventh subject (No. 10), the band is lighter than expected (Fig. 6).

The two zinc finger Y–negative subjects seem to have a more extensive deletion involving both Y long and short arms leading to the formation of two minuscule fragments that did not stain on C-banding and were

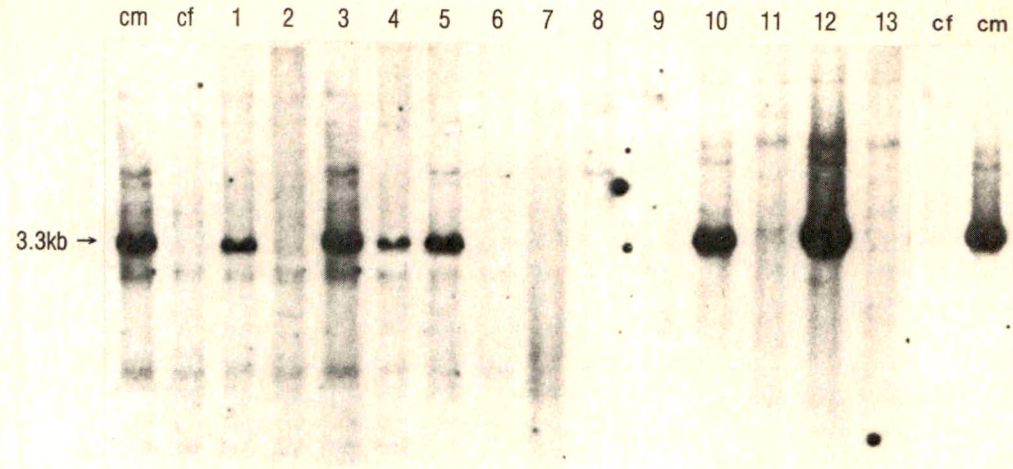


Fig. 5. Autoradiograph of *Eco*RI digest and 4B-2 probing of 13 study subjects. Y-specific 3.3 kb *Eco*RI band, marker of Yq11, is shown in control males *(cm)* and in six study subjects (Nos. 1, 3, 4, 5, 10, and 12). It is absent in control females *(cf)* and in remaining seven study subjects.

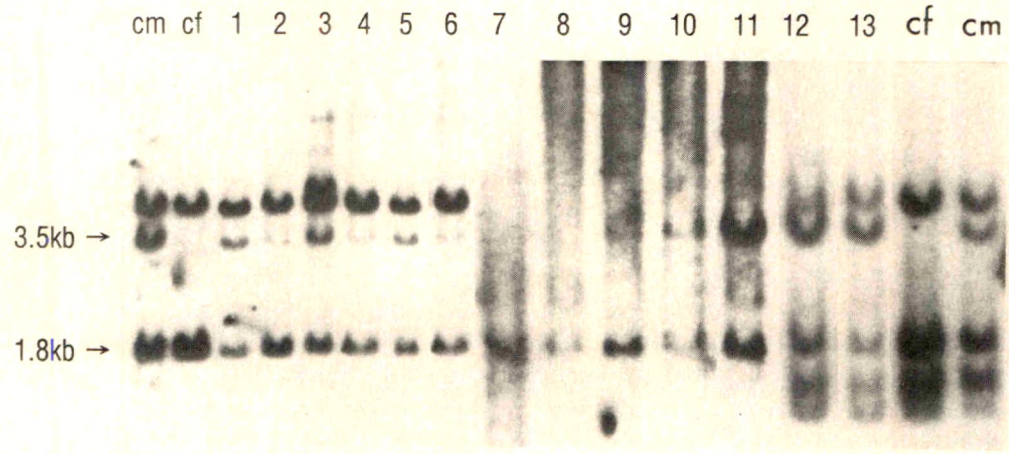


Fig. 6. Autoradiograph of *Eco*RI digest and pDP1007 probing of 13 study subjects. Y-specific 3.5 kb *Eco*RI (zinc finger Y) band is present in control males *(cm)* and 11 of 13 subjects with Y aneuploidy. It is absent in control females *(cf)* and in two subjects (Nos. 7 and 8) whose Y chromosomes are barely perceptible fragments. Phenotypically normal male (No. 13) and two typical Turner females (Nos. 11 and 12) have normal zinc finger Y bands.

thought to be "acentric" before molecular studies. Both subjects harbor bilateral streak gonads.

Among the 11 zinc finger Y–positive subjects, 7 were found to have only bilateral streak gonads; four had testicular differentiation ranging from bilateral scrotal testes with normal male phenotype (No. 13) to bilateral abdominal testes (No. 6) or unilateral testis (Nos. 2 and 3). The zinc finger Y band is normal in the subject with male phenotype (No. 13). It is almost normal in the subject with dic(Y) and unilateral abdominal testis (No. 3). However, the other two subjects (No. 2) with unilateral scrotal testis and (No. 6) with bilateral abdominal testes had a much reduced zinc finger Y band. Conversely among the seven zinc finger Y–positive subjects who harbor bilateral streaks, two had normal zinc finger Y bands (Nos 11 and 12), two a moderately reduced band (Nos. 1 and 5), and three had a significantly reduced zinc finger Y band (Nos. 4, 9, and 10).

Comment

Two thirds of XX males harbor on their paternal X, DNA material from the paternal Y chromosome short arm including the critical 140 kb region that is the candidate testicular determinant factor gene. Conversely, a few XY females with molecular deletions of Yp have been reported with Turner phenotype and

bilateral streak gonads.[20-22] The most conserved exon of the putative testicular determinant factor gene, represented by the zinc finger Y sequence (probe pDP1007), encodes a transcription protein with zinc finger domains.[1] The mechanism of testicular determination and the biochemical function of zinc finger Y are still unclear because it is unknown what these zinc fingers regulate. Cases of absence of Y sequences in 45,X mixed gonadal dysgenesis[6] and integrity of Y DNA in a nonmosaic 46,XY subject with unilateral streak and contralateral testis[5] would suggest that mosaicism alone cannot account for a complete or partial failure of testicular differentiation in several subjects with Y aneuploidy. One might argue that the gonadal phenotype depends on the relative prevalence of 46,XY cells versus 45,X cells in the gonad during embryonic development. One might also argue that the gonadal phenotype in typical mixed gonadal dysgenesis may theoretically reflect the relative prevalence of the 45,X cell line in the adult streak gonad versus the prevalence of the 46,XY cell line in the adult testis. Neither of these theories is supported by data and both would exclude Y chromosomal or Y gene mutations as the primary cause of abnormal gonadal differentiation. The large number of cytologically abnormal Y chromosomes in these subjects supports Y molecular rearrangements and makes one suspect Y gene mutations.

This is the first report of which we are aware that assesses the molecular integrity of Y DNA in a large series of subjects with gonadal dysgenesis with differing segments of the Y chromosome conserved and correlates the conserved segments with the presence of zinc finger Y. All 13 subjects harbor the Y centromere and Yp up to interval 3 (pDP105/A) and Yq down to distal interval 6 (pDP105/B) (Table I; Figs. 1, 3, and 4). Only two subjects lost the zinc finger Y sequence on distal Yp (Nos. 7, and 8) and both had only bilateral streak gonads (Table I, Fig. 6). No subject was identified as having testes and lacking the zinc finger Y sequence. All subjects (n = 4; Nos. 2, 3, 6, and 13) with unilateral or bilateral testes are positive for this sequence (Table I, Fig. 6). The phenotypically normal male with bilateral scrotal testes does have a normal zinc finger Y band (No. 13), whereas one of the three sexually ambiguous subjects shows almost a normal zinc finger Y band (No. 3). However, the remaining two sexually ambiguous subjects exhibit reduced zinc finger Y bands (Nos. 2 and 6). Conversely, the seven subjects with complete failure of testicular differentiation and bilateral streak gonads (Nos. 1, 4, 5, 9, 10, 11, and 12) are also positive for the zinc finger Y sequence. It is intriguing that four of these subjects shows moderate size (Nos. 1 and 5) to normal size (Nos. 11 and 12) zinc finger Y bands and they still failed to have any testicular development. For the remaining three (Nos. 4, 9, and 10) the zinc finger Y bands are as reduced as for the subject with unilateral scrotal testis (No. 2) and the subject with bilateral abdominal testes (No. 6).

The lack of testicular development in subjects who carry the sequence suspected to be testicular determinant factor may be attributed to quantitative or qualitative factors. The quantitative factor involves a critical threshold of genetically normal Y-bearing cells during early embryogenesis. The qualitative factor is related to qualitative changes in structural or regulatory genes involved in the testicular differentiation pathway. It is almost impossible to assess the quantitative factor. This report, which shows the complete lack of testicular development despite the presence of apparently adequate amounts of zinc finger Y sequence, and, conversely, testis development despite significantly reduced zinc finger Y, suggests that mosaicism per se may not completely account for lack of testicular development. This assumption is further supported by sporadic case reports of nonmosaic 46,XY mixed gonadal dysgenesis with all Y DNA sequences preserved.[5] The lack of testicular development in this situation should raise the possibility that point mutations, frame shift mutations, not detectable by Southern analysis with pDP1007, may occur in the structural and regulatory portions of the testicular determinant factor gene, vitiating testicular development.

Palmer et al.,[11] in a continuing evaluation of the Yp region, identified sequences distal to but not including the testicular determinant factor region of Page that are exclusively present in a small subset of zinc finger Y–negative XX males. Four of these 14 zinc finger Y–negative XX males were found positive for three Y-specific pseudoautosomal boundary probes. However, none of the four patients had the classic phenotype of XX males. Palmer et al. reaffirm the current controversy between the zinc finger Y locus by Page and the pseudoautosomal boundary region as the potential testicular determinant factor candidate. In the female withY;22 translocation and bilateral streak gonads used by Page for the isolation of testicular determinant factor, the 140 kb testicular determinant factor region is absent but the pseudoautosomal boundary region locus preserved. Palmer et al. excluded position polymorphism only on the basis of a population of 150 controls. This controversy only points out the need for further studies of both the candidate testicular determinant factor locus of Page and the pseudoautosomal boundary region locus of Palmer. Most importantly the zinc finger Y sequence of Page encodes a protein with zinc fingers that have DNA binding properties. The pseudoautosomal boundary region described by Palmer et al. is not known to encode protein products.

This report also provides the deletion map of a wide variety of rearranged Y chromosomes seen in the Y

aneuploidy syndrome. It is interesting that most break points are limited to distal Yq11 or to Yq12 and that the zinc finger Y locus is spared even in Yp deletions (Nos. 3 and 9). The most extensive deletions as in subjects Nos. 7 and 8 are associated with gonadal neoplasia. The comparison between molecular genotype and gonadotype in these subjects with a wide variety of altered Y chromosomes provides an important body of primary data for further investigations of the role of zinc finger Y and of the pseudoautosomal boundary region in testicular determination. Analysis of the DNAs of these subjects with different gonadal phenotypes with the use of different exons from both the putative testicular determinant factor region of Page and the pseudoautosomal boundary region of Palmer should be the subsequent steps to clarify the role of Y sequences in initiating human testicular differentiation.

We gratefully acknowledge Dr. David Page for providing the probes pDP105 and pDP1007, Dr. Robert Erickson for providing the probe Y97, and Dr. Kirby Smith for providing the probe 4B-2.

REFERENCES

1. Page DC, Mosler R, Simpson EM, et al. The sex determining region of the human Y chromosome encodes a finger protein. Cell 1987;51:1091-104.
2. McDonough PG, Tho SPT. The spectrum of 45,X/46,XY gonadal dysgenesis and its implications. Pediatr Adolesc Gynecol 1983;1:1-17.
3. McDonough PG, Tho SPT, Trill JJ, Byrd JR, et al. Use of two different deoxyribonucleic acid probes to detect Y chromosome deoxyribonucleic acid in subjects with normal and altered Y chromosomes. Am J Obstet Gynecol 1986;154:737-48.
4. Roach DJ, Tho SPT, Plouffe L, McDonough PG. Asymmetric gonadal dysgenesis in a 46,XY individual. Pediatr Adolesc Gynecol 1988;1:129-30.
5. Cantrell MA, Bicknell JN, Pagon RA, et al. Molecular analysis of 46,XY females and regional assignment of a new Y chromosome–specific probe. Hum Genet 1989; 83:88-92.
6. Waibel F, Scherer G, Fraccaro M, et al. Absence of Y-specific DNA sequences in human 46,XX true hermaphrodites and in 45,X mixed gonadal dysgenesis. Hum Genet 1987;76:332-6.
7. Weissenbach J, Goodfellow PN, Smith KD. Report of the committee on the genetic constitution of the Y chromosome. Cytogenet Cell Genet 1988;49:129-31.
8. Sinclair AH, Foster JW, Spencer JA, et al. Sequences homologous to ZFY, a candidate human sex determinating gene, are autosomal in marsupials. Nature 1988;336: 780-3.
9. Bull JJ, Hillis DH, O'Steen S. Mammalian ZFY sequences exist in reptiles regardless of sex-determining mechanism. Science 1988;242:567-9.
10. Kooper P, Gubbbay J, Collignon J, Lovel-Badge R. ZFY gene expression patterns are not compatible with a primary role in mouse sex determination. Nature 1989; 342:940-2.
11. Palmer MS, Sinclair AH, Berta P, et al. Genetic evidence that ZFY is not the testis determining factor. Nature 1989;342:937-9.
12. Vergnaud G, Page DC, Simmler MC, et al. A deletion map of the human Y chromosome based on DNA hybridization. Am J Hum Genet 1986;38:109-24.
13. Wolfe J, Darling SM, Erickson RP, et al. Isolation and characterization of an alphoid centromeric repeat family from the human Y chromosome. J Mol Biol 1985; 182:477-85.
14. Bernstein R, Rosendorff J, Ramsay M, et al. A unique dicentric X;Y translocation with Xq and Yp breakpoints: cytogenetic and molecular studies. Am J Hum Genet 1987;41:145-56.
15. Burk RD, Patrick MA, Smith KD. Characterization and evolution of a single copy sequence from the human Y chromosome. Mol Cell Biol 1985;5:576-81.
16. Maniatis T, Fritsch ER, Sambrook J. In: Molecular cloning: a laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1982:383.
17. Rigby PWJ, Dieckmann M, Rhodes C, Berg P. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translocation with DNA polymerase I. J Mol Biol 1987;113:237-51.
18. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1984;137:266-7.
19. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503-17.
20. Affara NA, Ferguson-Smith MA, Magenis RE, et-al. Mapping the testis determinants by an analysis of Y-specific sequences in males with apparent XX and XO karyotypes and females with XY karyotypes. Nucleic Acids Res 1987;15:7325-42.
21. Disteche CM, Casanova M, Saal H, et al. Small deletions of the short arm of the Y chromosome in 46,XY females. Proc Natl Acad Sci USA 1986;83:7841-4.
22. Levilliers J, Quack B, Weissenbach J, Petit C. Exchange of terminal portions of X and Y chromosomal short arms in human XY females. Proc Natl Acad Sci USA 1989; 86:2296-300.

Isolation and characterization of the gene from a human genome encoding 17β-estradiol dehydrogenase: A comparison of Jar and BeWo choriocarcinoma cell lines

Cecil A. Long, MD,[a] Gregory S. Bauer,[a, c] Mark E. Lowe, MD, PhD,[b] Arnold W. Strauss, MD,[b, c] and Michael J. Gast, MD, PhD[a]

St. Louis, Missouri

17-β-Estradiol dehydrogenase is required for the enzymatic interconversion of estradiol and its weaker related sex steroid, estrone. We isolated and sequenced a complementary deoxyribonucleic acid clone for 17β-estradiol dehydrogenase from the BeWo choriocarcinoma cell line. Comparison of the BeWo complementary deoxyribonucleic acid sequence to a previously derived placental complementary deoxyribonucleic acid sequence yields >98% homology. We also isolated the gene for 17β-estradiol dehydrogenase from the Jar human choriocarcinoma cell line and elucidated its primary nucleic acid structure. Significant differences in the Jar-deduced complementary deoxyribonucleic acid sequence clearly differentiate it from both the human placental and BeWo forms of 17β-estradiol dehydrogenase, indicating the existence of two genes for 17β-estradiol dehydrogenase in the human genome. Evaluation of 17β-estradiol dehydrogenase gene expression in BeWo and Jar cells was compared with expression in luteinized granulosa cells. Messenger ribonucleic acid for human placental 17β-estradiol dehydrogenase was identified in all three cell types as a 1.3 kilobase band on Northern blot analysis. A second messenger ribonucleic acid species measuring 2.1 kilobase was abundantly present in the granulosa cells. Whether these two species of messenger ribonucleic acid are involved in the regulation of the estradiol dehydrogenase genes is yet to be determined. (Am J Obstet Gynecol 1990;163:1976-81.)

Key words: 17β-Estradiol dehydrogenase, choriocarcinoma, estrogen metabolism

17β-Estradiol dehydrogenase (synonym, 17-ketosteroid oxidoreductase) occupies a pivotal position in the enzymatic cascade catalyzing the terminal step of estrogen biosynthesis. The enzyme is necessary for the reversible interconversion of estradiol and its weaker related steroid, estrone. It has also been suggested that the same enzyme may play a role in the interconversion of androstenedione and testosterone. 17β-Estradiol dehydrogenase may be involved in female well being in many widely varied areas including maintenance of bone and tissue integrity in menopause, control of the initiation of labor, uterine quiescence, and the ovarian-hypothalamic feedback system. In addition, 17β-estradiol dehydrogenase deficiency, although rare, can lead to male pseudohermaphroditism.[1]

From the Departments of Obstetrics and Gynecology,[a] Pediatrics,[b] and Biochemistry and Molecular Biophysics,[c] Washington University School of Medicine.

Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.

Reprint requests: Michael J. Gast, MD, PhD, Department of Obstetrics and Gynecology, Washington University School of Medicine, 4911 Barnes Hospital Plaza, St. Louis, MO 63110.

6/6/24718

The amino acid composition and biophysical properties of 17β-estradiol dehydrogenase have been elucidated through gel chromatography and active site topographic study with radioalkylated steroids and cofactors.[2-4] The enzyme exists as a 68,000-dalton dimer and uses pyridine nucleotides as cofactors. Activity of 17β-estradiol dehydrogenase has been shown in a wide variety of steroidogenic and nonsteroidogenic tissues; however, the most focused investigations have been with human placental 17β-estradiol dehydrogenase.

Specific activities of 17β-estradiol dehydrogenase from placenta and established gestational choriocarcinoma cell lines have been measured.[5] Although variable levels of 17β-estradiol dehydrogenase activity can be obtained in placental tissue, JEG-3, and BeWo cells, no 17β-estradiol dehydrogenase activity is noted in Jar cells. In addition, Western blot analysis has failed to show the presence of immunodetectable placental 17β-estradiol dehydrogenase-type enzyme in Jar cells.[5] To our knowledge, no mechanism has been established to explain the enzyme deficiency in the Jar cell line.

Three differing human placental 17β-estradiol dehydrogenase complementary deoxyribonucleic acid (cDNA) clones, including our own,[6-8] have previously

been isolated and sequenced. Sequence comparison of the clones reveals >95% homology. In situ hybridization studies[7] suggest that the human gene encoding 17β-estradiol dehydrogenase is located on the long arm of chromosome 17. The present investigation was undertaken to isolate and characterize the gene encoding 17β-estradiol dehydrogenase from a human genomic library, as well as define and compare gene expression in tumor cell lines with that of normal cells.

Methods

Isolation and DNA sequence analysis of BeWo cDNA and Jar genomic DNA. A cDNA library derived from messenger ribonucleic acid (mRNA) isolated from BeWo cells was constructed in the bacteriophage vector λgt10. Initial screening of the library was performed with a phosphorus 32–labeled 1100 base pair cDNA fragment from a human placental 17β-estradiol dehydrogenase clone, which we previously isolated and characterized.[8] 17β-Estradiol dehydrogenase–bearing plaques were purified by repeated screening with a modified version of the technique of Wahl and Berger.[9] Several of the clones were then amplified, their phage DNA was subjected to restriction endonuclease digestion, and they were subcloned into the sequencing vector pGEM4Z (Promega, Inc., Madison, Wis.). The largest of these clones measured 1.4 kilobases (kb) and was used for sequencing and subsequent library screening procedures. Sequencing was performed by a modification of the dideoxy chain termination method of Sanger with T7 DNA polymerase (Pharmacia, Piscataway, N.J.). Universal and specific synthetic oligonucleotides were used as sequencing primers.

A human genomic DNA library derived from the Jar choriocarcinoma cell line was provided by Dr. Irving Boime (Washington University, St. Louis). The library was screened with an 1100 base pair BeWo choriocarcinoma cDNA probe derived by Bam H1 digestion of the 1.4 kb clone. Once again, labeled plaques were lifted and further purified by rescreening at progressively lower plaque densities until several homogenous positive phage populations were identified. Three clones were then amplified and their phage DNA subjected to multiple restriction enzyme digestion. Southern blot analysis with the complete BeWo cDNA as well as fragments from both its 5′ and 3′ ends suggested that the entire Jar 17β-estradiol dehydrogenase gene was present in a single 6.0 kb fragment restricted by the enzyme *Kpn* I. This *Kpn* fragment and a smaller 1.7 kb *Sac* fragment that also hybridized to the 5′ end of the BeWo clone were then subcloned and sequenced by dideoxy chain termination.

RNA isolation and Northern blot analysis. Two different human choriocarcinoma cell lines, BeWo and Jar, were obtained from the American Type Culture Collection. Both of these gestational choriocarcinoma lines were developed from metastatic choriocarcinoma after a normal term birth.[10, 11] The cells were cultured in Dulbecco's modified Eagle's medium enriched with 15% fetal calf serum. Luteinized granulosa cells were obtained from follicle aspirations of patients undergoing ovarian hyperstimulation for in vitro fertilization.

Total RNA was isolated from BeWo, Jar, and granulosa cells by single-step guanidinium thiocyanate-chloroform extraction.[12] Fifteen micrograms of total RNA from each sample was separated on a 1.5% agarose–0.66 molar formaldehyde denaturing gel and transferred to a Gene Screen membrane (Biotechnology Systems NEN Research Products, Boston, Mass.). Overnight hybridization at 42° C was performed with a ^{32}P-labeled 1100 base pair BeWo fragment. The blot was then taken through a series of washes of increasing stringency with the final wash in 0.1X standard saline citrate/0.1% sodium dodecylsulfate at 65° C for 1.5 hours. Membrane filters were autoradiographed on Kodak X-omat film for up to 24 hours.

High specific activity 5′-α^{35}S-deoxyadenosine triphosphate (>1000 Ci/mmol) and 5′-α^{32}P-deoxycytidine triphosphate (>3000 Ci/mmol) were obtained from Amersham (Amersham, United Kingdom). Plasmid pGEM4Z was obtained from Promega. All reagent grade chemicals were obtained from Sigma Chemical (St. Louis).

Results

Isolation and characterization of human BeWo 17β-estradiol dehydrogenase cDNA. The screening of 0.6×10^6 plaques from a cDNA library derived from a human BeWo choriocarcinoma cell line with an 1100 base pair human placental 17β-estradiol dehydrogenase cDNA probe yielded 15 positive signals. Southern blot analysis confirmed that the cDNA sequence from the BeWo cell line corresponding to the human placental cDNA was contained in a 1.3 kb *Eco*RI fragment (data not shown). The 1.3 kb insert from this cDNA clone was isolated and the entire nucleotide sequence was determined (Fig. 1). Comparison of the BeWo 17β-estradiol dehydrogenase cDNA with the placental cDNA sequence established previously[8] revealed >98% homology, with complete homology in the substrate and cofactor binding domains.

Isolation and characterization of human Jar 17β-estradiol dehydrogenase genomic clone. Screening of 0.6×10^6 plaques from a Jar choriocarcinoma genomic library generated three positive signals. A single 6.0 kb *Kpn* I fragment contained the entire cDNA sequence

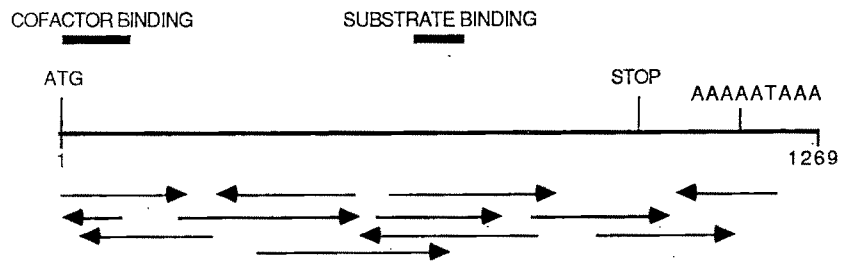


Fig. 1. Sequencing strategy for the BeWo 17β-estradiol dehydrogenase cDNA clone. Sequencing was carried out by a modified dideoxy nucleotide technique with ^{35}S-labeled deoxyadnenosine triphosphate. Universal primers and specific synthetic oligonucleotides were used as primers for sequencing reactions. The relative locations of steroid and cofactor binding sites and polyadenylation site are identified.

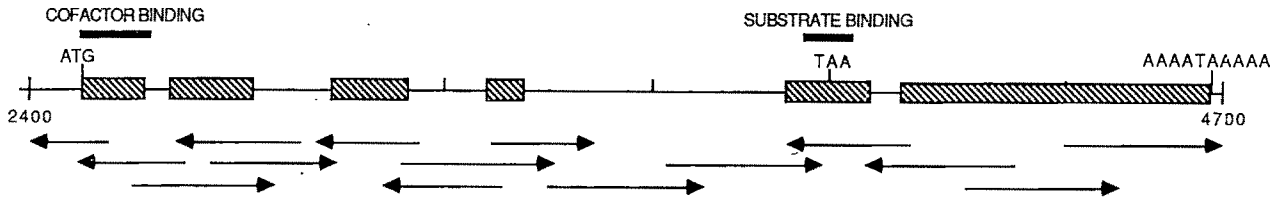


Fig. 2. Characterization and sequencing for the Jar 17β-estradiol dehydrogenase gene. The six encoding exons are represented by *striped boxes*. Nontranscribed regions are represented by *solid lines*. Relative locations of the substrate and cofactor binding sites are identified. ATG start codon and premature TAA stop codon are also noted.

of the BeWo 17β-estradiol dehydrogenase as determined by Southern blot analysis (data not shown). This *Kpn* fragment was placed into the plasmid vector pGEM4Z and sequenced by dideoxy chain termination. The human gene encoding for 17β-estradiol dehydrogenase contains at least six exons and spans approximately 2.4 kb of human genomic DNA (Fig. 2). The protein-encoding exons range in size from 72 to 555 base pair, whereas introns vary in length from 70 to 497 base pair. The nucleotide sequences surrounding intron-exon junctions correspond to those predicted by cDNA comparison, as well as published consensus sequences.[13] Each donor splice site begins with GT, whereas each acceptor splice site ends with AG. A consensus polyadenylation sequence (AATAAA), which delineates the 3′ end of the sixth and final exon of this gene, is also identified.

A composite nucleotide sequence representing the putative Jar cDNA encoding 17β-estradiol dehydrogenase was deduced from the genomic sequence and was compared with the BeWo choriocarcinoma cDNA (Fig. 3). Differences between the BeWo cDNA and Jar-deduced cDNA fell into three general categories. The first category included random point changes that were seen throughout the cDNA sequences. Second, two major deletions were noted in the Jar-deduced cDNA, a 9 base pair deletion within exon 3 and a 24 base pair deletion corresponding to the midportion of exon 6. The third important difference was a single point change from cytosine to thymine midway through exon 5, which would act to produce an in-frame TAA stop codon in the Jar gene product.

RNA blot hybridization analysis. To perform Northern blot analysis for the evaluation of transcription of the 17β-estradiol dehydrogenase gene in the Jar cell line, total RNA was extracted from BeWo and Jar cells as described in the Methods section. For a noncultured, nonneoplastic control, RNA from luteinized granulosa cells was also extracted and analyzed. The Northern blot was probed with the previously described 1100 base pair fragment constructed from the 3′ end of the BeWo cDNA (Fig. 4). A single dark band was found in the BeWo lane corresponding to an RNA of approximately 1.3 kb, which is identical to results obtained when analyzing placental RNA. In the Jar lane, a similar 1.3 kb RNA is also present. Slightly reduced density of this signal in the Jar lane may reflect modestly decreased homology of the Jar mRNA to the placental RNA. Two radiolabeled bands are noted in the granulosa cell lane—one corresponds to the placental 17β-estradiol dehydrogenase RNA of 1.3 kb and the second corresponds to a larger homologous RNA of 2.1 kb.

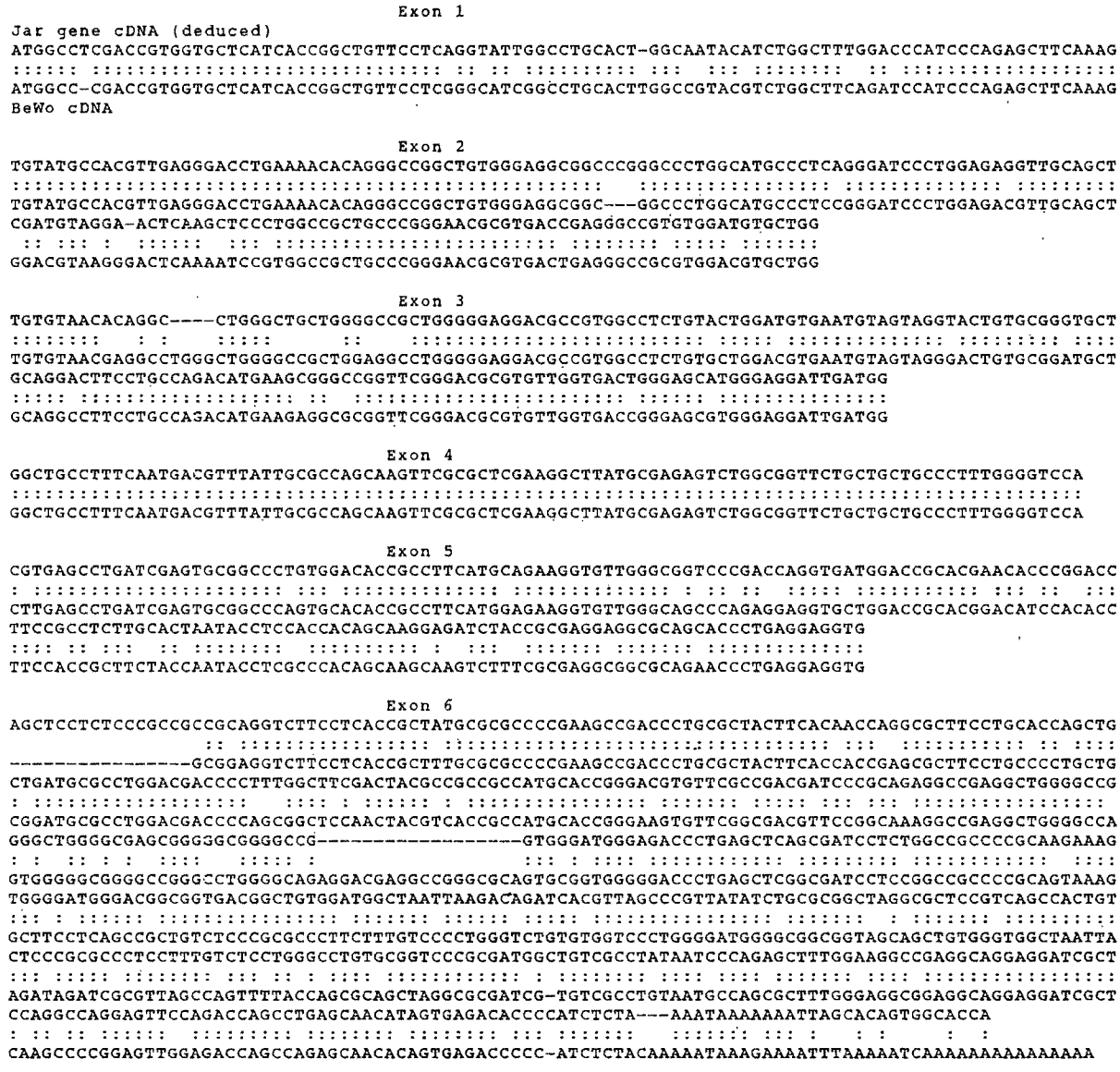


Fig. 3. Comparison of the nucleotide sequence of the BeWo cDNA encoding 17β-estradiol dehydrogenase *(bottom)* with the cDNA sequence deduced from the 17β-estradiol dehydrogenase gene from a Jar cell line *(above)*. Assignment of putative exons was made on the basis of locations of consensus donor and acceptor splice sequences and the known sequence of BeWo and placental cDNAs. *Colons* between sequences indicate identity of bases at respective positions in the sequence.

Comment

We isolated and sequenced a cDNA clone encoding 17β-estradiol dehydrogenase from the BeWo choriocarcinoma cell line. A genomic DNA sequence arising from DNA isolated from the Jar choriocarcinoma cell line has also been established. This gene consists of six exons and spans approximately 2.4 kb of genomic DNA. By comparing the BeWo cDNA with Jar genomic DNA, we were able to deduce a Jar cDNA encoding 17β-estradiol dehydrogenase. The intron-exon splice junctions predicted by this data and by the homology of the Jar cell line to the human placental cDNA are in complete agreement with the AG-GT rule.[13]

On alignment of the BeWo cDNA with the Jar-deduced cDNA there is approximately 77% nucleic acid homology between the two putative mRNA species. Differences between the two may be grouped into three categories: nonsignificant point differences, segmental deletions, and a point change from cytosine to thymine within a single critical codon in exon 5. When these

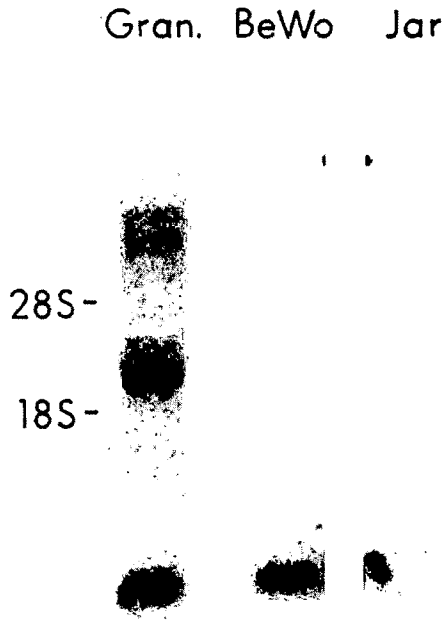


Fig. 4. DNA/RNA hybridization (Northern blot) analysis of luteinized granulosa, BeWo, and Jar cells. The Northern blot was probed with a ^{32}P-labeled 1100 base pair cDNA fragment from 3′ end of a BeWo cDNA clone. The lowest molecular weight band, present in all three cell types, represents a 1.3 kb DNA fragment. There is a second 2.1 kb species of mRNA clearly identified in the granulosa lane. Higher molecular weight material present in the granulosa cell lane likely represents artifact.

two sequences are translated into their respective amino acid sequences, the Jar-deduced cDNA has an in-frame stop codon rather than the glycine encoded by the BeWo cDNA. Of greatest significance, this termination signal would occur in the middle of the substrate binding domain of the Jar 17β-estradiol dehydrogenase enzyme. This pattern of mutations has been described in a variety of paired genes in which the phenomenon of gene complementation has occurred, including the gene for the human steroid-converting enzyme 21-hydroxylase.[14-16] On the basis of this data we hypothesized that two genes for 17β-estradiol dehydrogenase exist in the human genome but that only one of these genes (the complement) survived intact into the genome of the Jar cell line. The et al.[17] recently identified two apparently complementary tandemly arranged 17β-estradiol dehydrogenase genes within the human genome, providing partial confirmation to this theory.

Evaluation of gene expression in the BeWo and Jar cell lines was performed by DNA-RNA hybridization analysis. Probing Northern blots of RNA extracts from both BeWo and Jar cells with an 1100 base pair fragment constructed from the cDNA of BeWo, we consistently found mRNA corresponding to 1.3 kb in each cell line. This is identical to results obtained when analyzing RNA.[8] In contrast, RNA extracts from luteinized granulosa cells exhibited not only a 1.3 kb signal, but also a larger, distinct RNA of 2.1 kb. These granulosa cells, although not cultured, were obtained from patients undergoing ovarian hyperstimulation via human menopausal gonadotropins and human chorionic gonadotropin. DNA-RNA hybridization analysis of BeWo cells under less stringent conditions also reveals this larger 2.1 kb mRNA, but as a minor component (data not shown). We have no explanation for the second form of 17β-estradiol dehydrogenase message, although it would be tempting to propose a regulatory role for what we may assume is an alternately spliced RNA product. Experiments are under way in our laboratory to evaluate the less likely possibility that one of these messages may also represent the transcription product of the complementary, inactive gene.

In summary, we compared the molecular biology of 17β-estradiol dehydrogenase in two choriocarcinoma cell lines, BeWo and Jar, with that of the human placenta. The data presented herein indicate that two genes for 17β-estradiol dehydrogenase exist in the human genome. The predicted amino acid sequence based on the BeWo cDNA is essentially identical to that predicted from the established placental cDNA. On the other hand, the deduced Jar cDNA has major differences, including a premature stop signal interrupting the predicted amino acid sequence in the area of the substrate binding site. This suggests that only a single gene for 17β-estradiol dehydrogenase has survived intact into the Jar genome, that this gene does not produce an enzymatically active protein product, and that the absence of an intact complementary gene may account for the lack of 17β-estradiol dehydrogenase activity in this choriocarcinoma cell line.

We acknowledge Patricia B. McEwen and Harold F. Sims for their continuing encouragement and superior technical assistance.

REFERENCES

1. Eckstein B, Cohen S, Farkas A, Rosler A. The nature of the defect in familial male pseudohermaphroditism in Arabs of Gaza. J Clin Endocrinol Metab 1989;68:477-85.
2. Murdock GL, Chin CC, Offord RE, Bradshaw RA, Warren JC. Human placental 17β-estradiol dehydrogenase: identification of a single histidine residue affinity labelled by both 3-bromoacetoxyestrone and 12β-bromoacetoxy-4-estrene-3,17-dione. J Biol Chem 1983;258:11460-4.
3. Langer LJ, Engel LL. Human placental estradiol 17β dehydrogenase: concentration, characterization, and assay. J Biol Chem 1958;233:583-8.
4. Nicolas JC, Harris JI. Human placental 17β-oestradiol dehydrogenase: sequence of a tryptic peptide containing an essential cysteine. FEBS Lett 1973;29:173-6.
5. Milewich L, Shaw CB, Pruett KM, Mason JI. 17β hydroxysteroid oxidoreductase activity in choriocarcinoma cells in culture. In: Proceedings of the thirty-sixth annual meeting of the Society for Gynecologic Investigation 1989:192 [Abstract 526]. San Diego: Society for Gynecologic Investigation.
6. Peltoketo H, Isomaa V, Maentausta O, Vihko R. Complete

amino acid sequence of human placental 17β estradiol dehydrogenase deduced from cDNA. FEBS Lett 1988; 239:73-7.
7. The VL, Labrie C, Zhao HF, et al. Characterization of cDNAs for human estradiol 17β-dehydrogenase and assignment of the gene to chromosome 17: evidence of two mRNA species with distinct 5'-termini in human placenta. Molec Endocrinol 1989;3:1301-9.
8. Gast MJ, Sims HF, Murdock GL, Gast PM, Strauss AW. Isolation and sequencing of a cDNA clone encoding human placental 17β-estradiol dehydrogenase: identification of the putative cofactor binding site. AM J OBSTET GYNECOL 1989;161:1726-31.
9. Wahl GM, Berger SL. Screening colonies or plaques with radioactive nucleic acid probes. In: Berger SL, Kimmel AR, eds. Methods in enzymology: guide to molecular cloning techniques. San Diego, California: Academic Press, Inc, 1987:415-23.
10. Pattillo RA, Gey GO. The establishment of a cell line of human hormone-synthesizing trophoblastic cells in vitro. Cancer Res 1968;28:1231-6.
11. Pattillo RA, Ruckert A, Hussa R, Bernstein R, Delfs E. The Jar cell line—continuous human multihormone production and controls. In Vitro 1971;6:398-9.
12. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.
13. Breathnach R, Chambon P. Organization and expression of eucaryotic split genes coding for proteins. Ann Rev Biochem 1981;50:349-83.
14. White PC, Grossberger D, Onufer BJ, et al. Two genes encoding steroid 21-hydroxylase are located near the genes encoding the fourth component of complement in man. Proc Natl Acad Sci 1985;82:1089-93.
15. Harada F, Kimura A, Iwanaga T, Shimozawa K, Yata J, Sasazuki T. Gene conversion-like events cause steroid 21-hydroxylase deficiency in congenital adrenal hyperplasia. Proc Natl Acad Sci 1987;84:8091-4.
16. Fitch DHA, Mainone C, Goodman M, Slightom JL. Molecular history of gene conversions in the fetal γ-globulin genes. J Biol Chem 1990;265:781-93.
17. The VL, Labrie C, Simard J, et al. Structure of two in tandem human 17β-hydroxysteroid dehydrogenase genes. Mol Cell Endocrinol 1990;4:268-75.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1990 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 162 (January-June) and Vol. 163 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby–Year Book, Inc., Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 7351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Antiprogestin treatment decreases midluteal luteinizing hormone pulse amplitude and primarily exerts a pituitary inhibition

Donna Shoupe, MD,[a] Daniel R. Mishell, Jr, MD,[a] Gregory Fossum, MD,[a] Bradford L. Bopp,[a] Irving M. Spitz, MD,[b] and Rogerio A. Lobo, MD

Los Angeles, California, and New York, New York

Mifepristone (RU 486), a synthetic steroid with antiprogesterone receptor activity, was given with and without naloxone hydrochloride to six women in the midluteal phase to investigate the role of progesterone in the modulation of endogenous opioid activity and the secretion of luteinizing hormone and cortisol. Subjects were evaluated during four sequential monthly admissions during which multiple blood samples were obtained every 15 minutes for 8 hours. Patients were studied during a baseline cycle, after administration of RU 486 alone (100 mg/day), naloxone with RU 486, and naloxone alone. After administration of RU 486 there was a significant decline in total luteinizing hormone secretion ($p < 0.01$) and luteinizing hormone pulse amplitude ($p < 0.05$), but compared with baseline there was no significant change in luteinizing hormone pulse frequency. After infusion of naloxone there was a significant increase in mean luteinizing hormone values ($p < 0.05$) and luteinizing hormone pulse frequency ($p < 0.01$) but no change in pulse amplitude. There was no significant difference in mean luteinizing hormone values or luteinizing hormone pulse amplitude and frequency between administration of RU 486 and naloxone plus RU 486. Administration of naloxone alone, RU 486 alone, and RU 486 plus naloxone caused a significant increase in cortisol as compared with baseline cycles ($p < 0.05$). These data further support the notion that progesterone is important in the control of luteinizing hormone secretion and suggest that progesterone may primarily influence luteinizing hormone pulse amplitude and pituitary release of luteinizing hormone during the luteal phase. (Am J Obstet Gynecol 1990;163:1982-5.)

Key words: RU 486, pituitary effect

In a normal cycle, rising levels of progesterone in the luteal phase of the menstrual cycle is associated with a changing pattern of luteinizing hormone (LH) secretion. LH pulses change from being of high frequency and low amplitude during the follicular phase to pulses of high amplitude and low frequency during the luteal phase.[1] The frequency of LH pulses declines progressively during the luteal phase and correlates with the cumulative progesterone exposure.[1]

Mifepristone (RU 486), a synthetic steroid that binds to progesterone receptors and blocks the action of progesterone, appears to have multiple effects on the hypothalamic-pituitary-ovarian axis. When administered in the follicular phase of the menstrual cycle it blocks ovulation and results in a decline in estradiol[2, 3] and LH[4] levels during treatment. Addition of RU 486 to cultured human granulosa cells causes a decline in progesterone[5] secretion into the media whereas its administration in the midluteal phase in some subjects is associated with a decline both in LH levels[6, 7] and luteolysis.[8-10] In addition, RU 486 binds to the glucocorticoid receptor even more strongly than dexamethasone.[11] The antiglucocorticoid action of RU 486 appears to occur during periods of active adrenocorticotropic hormone (ACTH) secretion.[12, 13]

Endogenous opioids appear to play an important role in the determination of LH pulsatility by inhibiting hypothalamic gonadotropin-releasing hormone secretion.[14] This inhibitory action is thought to be modulated by ovarian steroids[15] and the maximum suppression occurs during the high-estrogen and high-progesterone phases of the cycle. Infusion of naloxone during the luteal phase in a normal subject results in the uncoupling of this inhibitory activity, an increase in LH pulse frequency, and a decline in pulse amplitude.[16]

Our purpose was to investigate the effects of an an-

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine,[a] and the Center for Biomedical Research, The Population Council.[b]

Supported in part by grants from the Andrew W. Mellon Foundation, George J. Hecht Fund, Rockefeller Foundation, and General Clinical Research Center program of the Division of Research Resources, National Institutes of Health (M01-RR-43 GCRC, DRR, NIH). Computational assistance was provided by the Clinfo Project, funded by the Division of Research Resources of the National Institutes of Health under Grant No. RR-00043.

Presented in part at the Thirty-third Annual Meeting of the Society for Gynecologic Investigation, Toronto, Ontario, March 19-22, 1986.

Reprint requests: Donna Shoupe, MD, Department of Obstetrics and Gynecology, University of Southern California School of Medicine, 1240 N. Mission Road, L-946, Los Angeles, CA 90033.

6/6/24782

tiprogestin given in the midluteal phase on LH pulsatility and cortisol secretion, and to investigate this interaction with and without opioid blockade.

Material and methods

Six women, ages 21 to 33 years, with cycle lengths that averaged 28 ± 2 days, were evaluated for six consecutive cycles. Each woman was within 20% of ideal body weight and had a documented serum progesterone level >5 ng/ml in the cycle before entry to this study. During the study months, blood was obtained on day 10 and daily thereafter until the LH surge was detected by daily radioimmunoassay. During all four study cycles, patients were admitted to the Clinical Research Center at 7:00 AM, 6 to 9 days after the LH surge, and blood samples were obtained every 15 minutes for 8 hours. Before the first study admission, no medication was given. During the following cycle, RU 486 at a dose of 50 mg twice a day (50 mg tablets, supplied by Roussel Uclaf, Romainville, France) was begun 3 days before admission. The last pill was taken in the morning of admission between 6:00 and 7:00 AM.

During the fourth cycle, RU 486 was administered as in cycle 2 with the addition of a naloxone infusion. During the sixth cycle, naloxone 2 mg/hour beginning at 8:00 AM was infused alone. Two baseline blood samples were obtained before the naloxone infusion. The third and fifth cycles were rest cycles, as the patients were not given medication and no blood sampling occurred.

Blood samples were allowed to clot and sera were separated and stored at −20° C until assayed for LH, estradiol, progesterone, and cortisol by previously described methods.[2, 6] Intra- and interassay coefficients of variation of the LH assay were <6% and <12%, respectively. Cortisol was measured in two baseline samples 15 minutes apart, and then hourly. All samples from an individual subject were measured in a single assay.

Statistics. Pulse frequency and amplitude were calculated with a computer program[17] based on the criteria described by Santen and Bardin.[18] A pulse was considered significant if there was an increase in LH over the mean of two baseline values that exceeded twice the interassay coefficient of variation. Pulse amplitude was defined as the absolute difference in hormone concentrations between peak and nadir values.

Other statistical comparisons were carried out with the assistance of the Clinfo computer program with analysis of variance and the Student t test. Values are expressed as mean ± SE.

Results

Baseline progesterone and estradiol levels before infusion during the first cycle were 13.4 ± 4.5 ng/ml and 135.2 ± 15.6 pg/ml, respectively, and were not significantly different during the other treatment months (Table I).

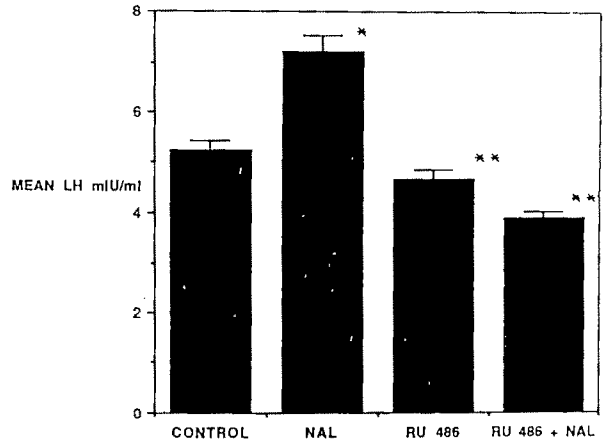


Fig. 1. Mean LH values ± SEM in following groups: control, naloxone *(NAL)* alone, RU 486, and RU 486 plus naloxone. *$p < 0.05$ from baseline; **$p < 0.05$ from other three groups.

Table I. Progesterone and estradiol levels before infusion

	Progesterone (ng/ml)	*Estradiol (pg/ml)*
Baseline	13.4 ± 4.5	135.2 ± 15.6
RU 486	14.0 ± 8.7	110.2 ± 48.3
RU 486 + naloxone	14.5 ± 8.6	136.0 ± 73.9
Naloxone	11.2 ± 10.1	111.3 ± 76.5

No significant differences.

When analyzed by analysis of variance, mean LH values during the sampling period were significantly lower after administration of RU 486 alone and RU 486 plus naloxone compared with baseline ($p < 0.05$) (Fig. 1). Mean LH values after infusion of naloxone alone (7.2 ± 0.3 mIU/ml) increased significantly ($p < 0.05$) compared with all other sampling periods. There was no difference in mean LH values between administration of RU 486 alone and RU 486 with naloxone.

During the baseline cycle there were 1.75 ± 0.2 LH pulses with a mean pulse amplitude of 5.2 ± 0.5 mIU/ml during the 8-hour sampling period. After administration of RU 486 there was a significant decline in LH pulse amplitude ($p < 0.05$), whereas no significant change occurred in LH pulse frequency as noted above. No significant changes occurred in LH pulse frequency in any group except with infusion of naloxone in which LH pulse frequency increased to 2.7 ± 0.4 ($p < 0.05$). The administration of RU 486 with naloxone also decreased LH pulse amplitude ($p < 0.05$), whereas increases in LH pulse amplitude were evident with infusion of naloxone alone (Table II).

Table II. LH pulsatility in 8 hours during four different treatment periods

	No. of pulses	*Pulse amplitude mIU/ml*
Baseline	1.75 ± 0.25	5.2 ± 0.5
RU 486	0.9 ± 0.2	3.1 ± 0.8*
RU 486 + naloxone	1.5 ± 0.3	3.0 ± 0.4*
Naloxone	2.7 ± 0.4*	6.5 ± 0.9

Mean ± SE.
*$p < 0.05$ compared with baseline.

Table III. Mean cortisol values during four sampling periods

	Cortisol µg/dl
Baseline	11.0 ± 1.5
RU 486	17.0 ± 1.7*
RU 486 + naloxone	16.0 ± 1.7*
Naloxone	16.0 ± 1.4*

*$p < 0.05$ compared with baseline.

Figs. 2 and 3 show the LH values in a typical patient during the control cycle, after treatment with RU 486 with and without naloxone and after infusion of naloxone alone.

Mean cortisol values were significantly higher than baseline in all treatment groups after RU 486 alone, RU 486 plus naloxone, and naloxone alone (Table III).

Comment

Administration of RU 486 before ovulation in normally cycling women causes ablation of the normal LH–follicle-stimulating hormone surge with lowering of serum estradiol and progesterone.[2] When given in the midluteal phase, high doses of RU 486 (between 200 and 800 mg) cause luteolysis and a decline in estradiol within 24 hours in up to 35% of subjects.[5] In this study, treatment in the midluteal phase with 100 mg RU 486 for 3 days resulted in no significant changes in estradiol or progesterone.

Because progesterone has been shown to increase LH pulse amplitude and decrease the gonadotropin-releasing hormone–mediated pulses in the luteal phase of the normal menstrual cycle,[19] administration of an antiprogestin in the midluteal phase would be expected to decrease LH pulse amplitude and increase pulse frequency. In this study, there was a decline in LH pulse amplitude and mean LH secretion after administration of RU 486. However, we noted a decrease in LH pulse frequency after RU 486, although this was not significant. This discrepancy may be a result of the inherent difficulties involved in accurately detecting the presence of LH pulses when LH levels are low. Interpretation of LH pulse frequency data is also limited because of the low number of pulses that occur in the luteal phase. Previous work is in agreement with our findings in that a slight but nonsignificant increase in LH pulse frequency was found to occur along with a significant decline in pulse amplitude after administration of RU 486 in the midluteal phase.[7] Alternatively, these data may suggest that progesterone exerts a primary influence on LH pulse amplitude and the failure to detect changes in LH pulse frequency signifies the lack of a direct hypothalamic effect during the luteal phase.

It is most plausible that the changes in LH secretion and LH pulse amplitude, specifically, are a result of a direct pituitary effect of RU 486. This finding is sup-

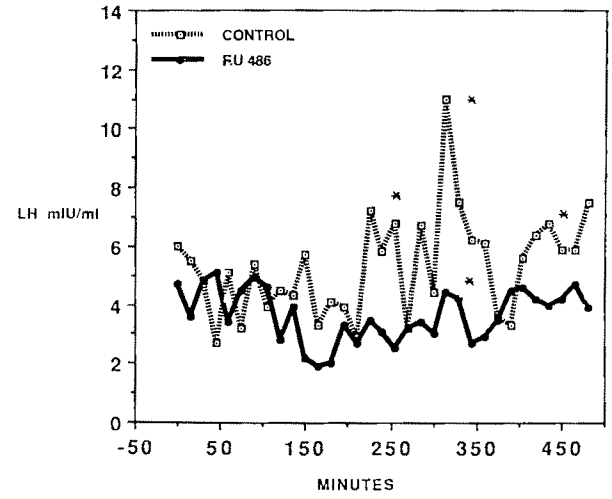


Fig. 2. LH values in typical subject during 8 hours in control cycle and after administration of RU 486. *Indicates pulses.

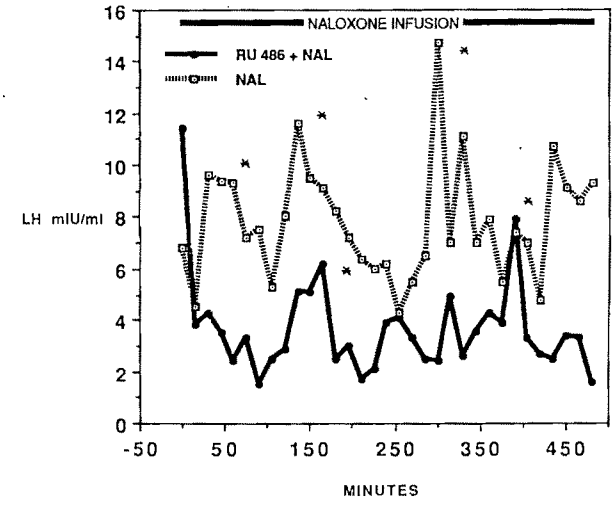


Fig. 3. LH values in typical subject during 8-hour naloxone *(NAL)* infusion with and without prior administration of RU 486. *Indicates pulses.

ported by previous work that shows that RU 486 impairs the pituitary response to gonadotropin-releasing hormone.[7, 20] RU 486 alone did not inhibit LH pulse frequency, suggesting that progesterone may not have a direct influence on hypothalamic gonadotropin-releasing hormone discharge. Inasmuch as naloxone alone (opioid blockade) increased LH pulse frequency, this natural opioid effect may be in part independent of the effect of progesterone. Results of administration of RU 486 in combination with naloxone were not different from those of RU 486 when used alone.

The antiglucocorticoid effect of RU 486 is well known[11] and the increase in cortisol after administration of RU 486 is thought to be mediated by increases in ACTH.[11] The increase in cortisol after administration of naloxone alone has also been reported by our group.[21] There is evidence that β-endorphin may regulate cortisol secretion by attenuating the cortisol response to exogenous ACTH and may also decrease the pituitary secretion of ACTH.[22, 23] However, the addition of naloxone and RU 486 were neither additive nor inhibitory to cortisol, suggesting that these two mechanisms of increasing ACTH and cortisol release may be independent.

These data support a direct pituitary action of RU 486 in decreasing LH pulse amplitude and further suggest that the effect of progesterone on LH pulse amplitude may not be mediated through endogenous opioids. These data further suggest that the inhibitory hypothalamic effect of opioid peptides may not be fully dependent on progesterone.

REFERENCES

1. Filicori M, Butler JP, Crowley WF Jr. Neuroendocrine regulation of the corpus luteum in the human. J Clin Invest 1984;73:1638.
2. Shoupe D, Mishell DR Jr, Page MA, Madkour H, Spitz IM, Lobo RA. Effects of the antiprogestin RU 486 in normal women. II. Administration in the late follicular phase. Am J Obstet Gynecol 1987;157:1421-6.
3. Stuenkel CA, Garzo VG, Morris S, Liu JH, Yen SSC. Effects of the antiprogesterone RU 486 in the early follicular phase of the menstrual cycle. Fertil Steril 1990;53:642.
4. Permezel JM, Lenton EA, Roberts I, Cooke ID. Acute effects of progesterone and the antiprogestin RU 486 on gonadotropin secretion in the follicular phase of the menstrual cycle. J Clin Endocrinol Metab 1989;68:960.
5. Dimattina M, Albertson B, Seyler DE, Loriaux DL, Falk FJ. Effect of the antiprogesterone RU 486 on progesterone production by cultured human granulosa cells: inhibition of the ovarian 3β-hydroxysteroid dehydrogenase. Contraception 1986;34:199.
6. Shoupe D, Mishell DR Jr, Lahteenmaki P, et al. Effects of the antiprogestin RU 486 in normal women. I. Single-dose administration in the midluteal phase. Am J Obstet Gynecol 1987;157:1415-20.
7. Garzo VG, Liu J, Ulmann A, Baulieu E, Yen SSC. Effects of an antiprogesterone (RU 486) on the hypothalamic-hypophyseal-ovarian-endometrial axis during the luteal phase of the menstrual cycle. J Clin Endocrinol Metab 1988;66:508.
8. Herrmann W, Wyss R, Riondel A, et al. Effect d'un steroide antiprogesterone chez la femme: interruption du cycle menstrual et de la grossesse andebut. C R Acad Sci (D) (Paris) 1982;294:933.
9. Schaison G, George M, Lestrat N, Reinberg A, Baulieu E-E. Effects of the antiprogesterone steroid RU 486 during midluteal phase in normal women. J Clin Endocrinol Metab 1985;61:484.
10. Nieman LK, Choate TM, Chrousos GP, et al. The progesterone antagonist RU 486: a potential new contraceptive agent. N Engl J Med 1987;316:187.
11. Healy DL, Chrousos GP, Schulte HM, Gold PW, Hodgen GD. Increased adrenocorticotropin, cortisol, and arginine vasopressin secretion in primates after the antiglucocorticoid steroid RU 486: dose-response relationships. J Clin Endocrinol Metab 1985;60:1.
12. Bertagna X, Bertagna C, Laton JP, Husson JM, Girard F. The new steroid analog 486 inhibits a glucocorticoid action in man. J Clin Endocrinol Metab 1984;59:25.
13. Gaillaid RC, Roindel A, Muller AF, Herrmann W, Baulieu EE. RU 486: a new steroid with antiglucocorticoid steroid that only disinhibits the human pituitary-adrenal system at a specific time of day. Proc Natl Acad Sci 1984;1:3879.
14. Rasmussen DD, Liu JH, Yen SSC. Endogenous opioid regulation of GnRH release from the human mediobasal hypothalamus (MBH) in vitro. J Clin Endocrinol Metab 1983;57:881.
15. Shoupe D, Lobo RA. The effects of estrogen and progestin on endogenous opioid activity in oophorectomized women. J Clin Endocrinol Metab 1985;60:178.
16. Quigley ME, Yen SSC. The role of endogenous opiates on LH secretion during the menstrual cycle. J Clin Endocrinol Metab 1980;51:179.
17. Kletzky OA, Fossum GT. Development of a simplified and flexible computerized pulse analysis program [Abstract 74]. In: Proceedings of the Endocrine Society annual meeting. New Orleans, Louisiana: Endocrine Society, 1988.
18. Santen RJ, Bardin CW. Episodic hormone secretion in man; pulse analysis, clinical interpretation, and physiologic mechanisms. J Clin Invest 1972;52:2617.
19. Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S, Bremner WJ. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. J Clin Endocrinol Metab 1984;58:378.
20. Liu J, Garzo G, Morris S, Stuenkd C, Ulmann A, Yen SSC. Disruption of follicular maturation and delay of ovulation after administration of the antiprogesterone RU 486. J Clin Endo Metab 1987;65:1135-40.
21. Shoupe D, Mishell DR Jr, Lobo RA. Evidence that oxytocin increases cortisol secretion and decreases LH pulsatility in normal women [Abstract 216]. In: Proceedings of the thirty-fifth annual meeting of the Society for Gynecologic Investigation. Baltimore, Maryland: Society for Gynecologic Investigation, 1987.
22. Taylor T, Dluhy RG, Williams GH. β-Endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. J Clin Endocrinol Metab 1983;57:592.
23. Beyer HS, Parker L, Li CH, Stuart D, Sharp BM. β-Endorphin attenuates the serum cortisol response to exogenous adrenocorticotropin. J Clin Endocrinol Metab 1986;62:808-11.

Luteal insufficiency: Correlation between endometrial dating and integrated progesterone output in clomiphene citrate–induced cycles

Bryan R. Hecht, MD,[a] Wadi A. Bardawil, MD,[c] Firyal S. Khan-Dawood, PhD,[b] and M. Yusoff Dawood, MD[b]

Chicago, Illinois, and Houston, Texas

Midluteal phase endometrium was histologically dated with midcycle luteinizing hormone surge time in 29 cycles from 10 parous women during untreated cycles (control) and treatment with clomiphene citrate 50 mg and 150 mg daily on days 5 through 9. Integrated progesterone output for 7 days after luteinizing hormone surge calculated from the daily plasma progesterone levels was 66.6 ± 9.8 ng/ml in the control group compared with 117.5 ± 18.6 ng/ml for clomiphene citrate 50 mg treatment and 152.1 ± 11 ng/ml for clomiphene citrate 150 mg treatment ($p \leq 0.05$). Only one cycle (clomiphene citrate 150 mg) had an out-of-phase endometrium and a significantly reduced integrated progesterone output of 28 ng/ml. All other cycles showed synchronous endometrial maturation. We conclude that luteal insufficiency as a result of clomiphene citrate treatment in ovulatory women is infrequent and is more likely to be a result of functional outcome of a relative lack of luteal phase progesterone output. (AM J OBSTET GYNECOL 1990;163:1986-91.)

Key words: Clomiphene citrate, luteal phase defect, progesterone, endometrial dating, endometrial asynchrony

Luteal phase defect and conception failure occur in some cycles in which clomiphene citrate has been administered for induction or augmentation of ovulation.[1, 2] The incidence and mechanisms of clomiphene citrate–induced luteal phase defect remain debatable. Deficient luteal progesterone production or a direct adverse effect of clomiphene on endometrial responsiveness are both mechanisms that could be responsible for producing this clinical phenomenon.

Histologic diagnosis of luteal phase defect is usually made by comparison of the actual endometrial histologic findings with those expected on the basis of the onset of menses after the biopsy.[3] Both clinical and histologic evidence suggest that use of this "next menses" criteria may not provide the most precise relationship to the actual time of ovulation. Thus, imprecise interpretation of the endometrial histology can occur with such criteria because the true postovulatory luteal age is not known. The use of the midcycle luteinizing hormone surge is a superior reference point for histologic dating of the endometrium.[4] To further define the effect of clomiphene citrate on luteal phase physiology, we conducted a controlled study in normal ovulatory women to compare (1) the actual luteal histology with the expected findings on the basis of the midcycle serum luteinizing hormone (LH) surge as well as with integrated progesterone output on the basis of daily plasma progesterone levels, and (2) the effect of high and low doses of clomiphene citrate on luteal phase endometrial development.

From the Department of Obstetrics and Gynecology, Northwestern University Medical School,[a] the Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School at Houston,[b] and the Department of Pathology, University of Illinois College of Medicine.[c]

Supported in part by the American College of Obstetricians and Gynecologists–Mead Johnson Fellowship for Clinical Research and the American College of Obstetricians and Gynecologists, District 6, Illinois Section.

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: M. Yusoff Dawood, MD, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School at Houston, 6431 Fannin, Suite 3.110, Houston, TX 77030.

6/6/24731

Material and methods

The study was approved by the Institutional Review Board of the University of Illinois at Chicago, where the study was conducted. Ten normal, fertile women with regular ovulatory menstrual cycles volunteered to participate and gave informed consent. All subjects had previously undergone tubal ligation or were using barrier contraceptive methods. The clinical characteristics of these subjects and treatment cycles have been reported by us in previous publications.[5, 6] The mean age was 30.3 ± 1.1 years (±SEM), weight was 77.3 ± 5.4 kg, parity was 2.4 ± 0.4, and the normal control menstrual cycle length was 28.5 ± 1.1 days.

Medication protocol. Eight subjects were studied for three consecutive menstrual cycles whereas two were studied for three nonconsecutive cycles. The first cycle

was a control cycle during which the subjects did not receive medication. In the second cycle, 50 mg clomiphene citrate was given daily on days 5 through 9 of the cycle and in the third cycle, 150 mg clomiphene citrate (50 mg three times a day) was given on days 5 through 9.

Basal body temperature was recorded by each subject for all cycles studied. The first-voided urine samples were collected daily, beginning on cycle day 11 and continued until an LH surge was identified or a sustained basal body temperature rise was evident. Daily peripheral heparinized venous blood samples were obtained between 8:00 AM and 10:00 AM beginning on cycle day 11. The plasma was separated and stored at −20° C until assayed. Plasma LH was measured by a specific double-antibody radioimmunoassay.[7] Plasma progesterone was measured by a specific radioimmunoassay as previously described with an antibody raised against progesterone-11-oxime bovine serum albumin conjugate.[8] To reduce interassay variability, all blood samples from the same patient were performed in the same assay for each hormone.

Endometrial biopsy specimens were obtained 8 to 12 days after the urine LH surge. Subsequently when the plasma LH levels were determined, midcycle plasma LH surge to endometrial biopsy intervals were 7 to 12 days, with a mean interval of 10.4 ± 0.2 days for the control cycle, 10.7 ± 0.4 days for the 50 mg clomiphene citrate treatment cycle and 10.8 ± 0.1 days for the 150 mg clomiphene citrate cycle. Ibuprofen 800 mg was given orally 1 hour before the endometrial biopsy, which was performed under a paracervical block. The uterine cavity was systematically and thoroughly curetted to obtain as much tissue as possible. Endometrial tissue was immediately rinsed in normal saline solution to remove blood and debris and a random piece was taken for histologic studies. The remaining tissue was used for steroid hormone receptor measurements.[5] Endometrial tissue was processed for routine hematoxylin and eosin staining and was subsequently randomly read and interpreted by a gynecologic pathologist (W.A.B.) who was unaware of the treatment given. Histologic dating was performed according to the criteria of Noyes et al.,[3] but the interval from the midcycle plasma LH surge to the day of biopsy (not the onset of next menses) was then used to calculate the expected dating.

Integrated progesterone values were calculated from the area under the curve of daily plasma progesterone with the trapezoidal rule for the first 7 days of the luteal phase (LH + 0 to LH + 7). Because the earliest biopsy performed in any cycle was on LH + 7, this 7-day interval was chosen to include the maximum number of days from surge to biopsy that could be compared in all cycles.

Data are expressed as mean ± SEM. Means were compared with analysis of variance and the nonpaired Student t test. A p value >0.05 was considered not significant.

Results

Clinical findings. All cycles (control and treatment) studied were ovulatory as determined by identification of an LH surge, biphasic basal body temperature recordings, elevated luteal phase plasma progesterone concentrations, and/or the presence of secretory endometrium.

Endometrial histology. Endometrium was not available for dating in one (clomiphene citrate 50 mg) of 30 cycles studied. All control (n = 10) and clomiphene citrate 50 mg cycles (n = 9) showed endometrial maturation within 48 hours of the chronologically expected date, calculated from the number of days after the LH surge on which the biopsy was obtained. One clomiphene citrate 150 mg treatment produced endometrium out of phase by 4 days (day 20 when day 24 was expected).

Integrated progesterone. In control cycles, integrated progesterone (mean ± SEM) was 66.6 ± 9.8 ng/ml (n = 7), compared with 117.5 ± 18.6 ng/ml (n = 8) for clomiphene citrate 50 mg and 152.1 ± 11 ng/ml (n = 8) for clomiphene citrate 150 mg treatment cycles (Fig. 1). In the clomiphene citrate 150 mg treatment cycle with out-of-phase endometrium, integrated progesterone was only 28 ng/ml. Fig. 2, *A* and *B* and Fig. 3, *A* and *B* show representative samples of in-phase and out-of-phase endometrium and their corresponding daily and integrated plasma progesterone values in two different women, each after treatment with 150 mg/day of clomiphene. According to customary criteria,[3] the histology in Fig. 2, *B* would be read as "day 23" in agreement with the expected histology on the basis of the LH surge (LH surge in an ideal cycle being day 13 and endometrial biopsy performed on LH + 10, making it day 23). The corresponding integrated progesterone value in this cycle was 146.9 ng/ml. The endometrial sample shown in Fig. 3, *B* was obtained on LH + 11 with an expected finding of "day 24." This biopsy was interpreted as "day 20," 4 days out of phase. The corresponding integrated progesterone value was only 28 ng/ml.

Thus only one of 19 cycles showed true luteal phase inadequacy during clomiphene citrate treatment (approximately 5%).

Comment

Because of the possibility that treatment with clomiphene citrate may directly or indirectly affect endometrial preparation and suitability for implantation, we examined the effect of low and high doses of clomiphene citrate on luteal phase endometrial histology

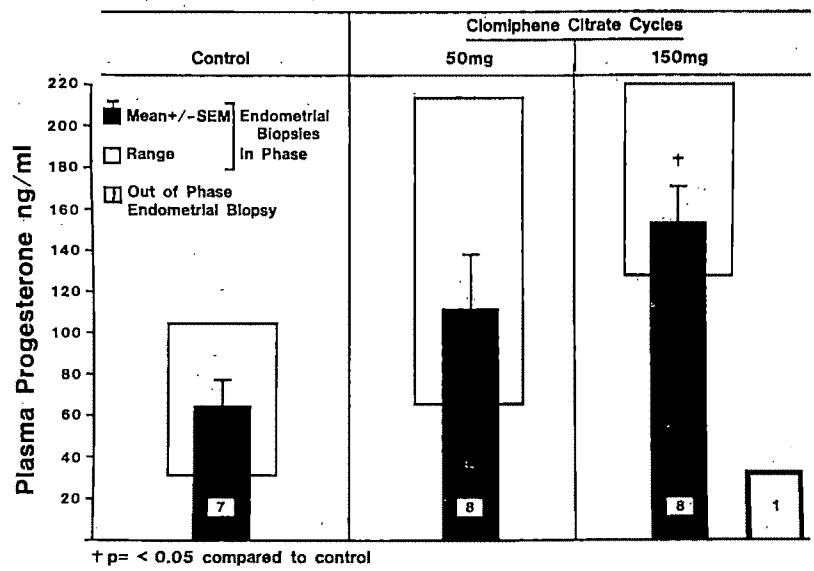


Fig. 1. Mean ± SEM integrated progesterone output for 7 days after midcycle plasma LH surge in control cycles (no treatment) and in clomiphene citrate 50 mg–treated and clomiphene citrate 150 mg–treated cycles in 10 parous women. Integrated progesterone output increased significantly during clomiphene citrate 150 mg treatment cycles ($p < 0.05$) but was significantly reduced in one cycle that had out-of-phase delayed endometrial histology.

in a group of normal ovulatory women. The use of normal ovulatory women should avoid other possible confounding factors that may cumulatively affect the endometrium in infertile women besides the use of clomiphene citrate. At a daily dose of 50 mg given from the fifth through the ninth day of the cycle, clomiphene citrate treatment did not affect histologic evidence of periimplantation phase endometrial maturation. However, at 150 mg daily, clomiphene citrate did produce one out-of-phase (delayed) endometrial biopsy with a corresponding low integrated progesterone output over the first 7 days of the luteal phase. In contrast, the remaining 150 mg clomiphene citrate–treated cycles had significantly higher integrated progesterone output compared with control cycles in the same women.

There is disagreement with regard to the sensitivity and reliability of endometrial dating by histology as described by Noyes et al.[3] Such dating is based on chronologic dating from the next menstrual period and assumes that all normal luteal phase lengths are 14 days—an assumption that is invalid.[9, 10] Only 27% of regular cycles had luteal phases of exactly 14 days whereas 31% had 12 days or fewer or 16 days or more.[10] The correlation of $r = 0.7$ between observers[4] and the interobserver variation of one day is also sufficient to cause a change in endometrial dating that would alter clinical management in 22% to 39% of the patients studied.[11] The intraobserver agreement of 24% and disagreement of >2 days in 10% of the same specimens read twice separately also attest to the subjectivity and variability of endometrial dating.[12] Furthermore, even when all the variables described in the technique of Noyes et al.[3] are used, Li et al.[12] have shown that the dating is imprecise and not as reliable as it is made out to be. Several investigators[9, 13, 14] have advocated the need to use morphometric analysis for the histologic dating to obtain more precise and valid interpretation. Only 5 of the 17 morphometric measurements examined have been found to be necessary for an accurate histologic dating.[13] The five predictive morphometric variables are the number of mitoses per 1000 gland cells, the amount of secretion in the gland lumen, the volume fraction of gland occupied by gland cell, the amount of pseudostratification of gland cells, and the amount of stromal predecidual reaction. We used the day of the plasma LH surge as the temporal reference point to estimate the expected histologic finding rather than the onset of the next menses. The timing of the LH surge is certainly more closely defined to the time of ovulation than to the next menstrual period and therefore will give a more precise and valid dating of the endometrium. Li et al.[4] found chronologic dating of the endometrium with the next menstrual period gave a larger variance than did use of midcycle serum LH, which reduced the incidence of out-of-phase endometrium from 42% to 10%.

Whereas endometrium obtained as late as possible in the cycle may have a greater degree of progesterone biologic effect and therefore is preferred by some pathologists for dating, others found the histologic features are more variable in the late luteal phase with

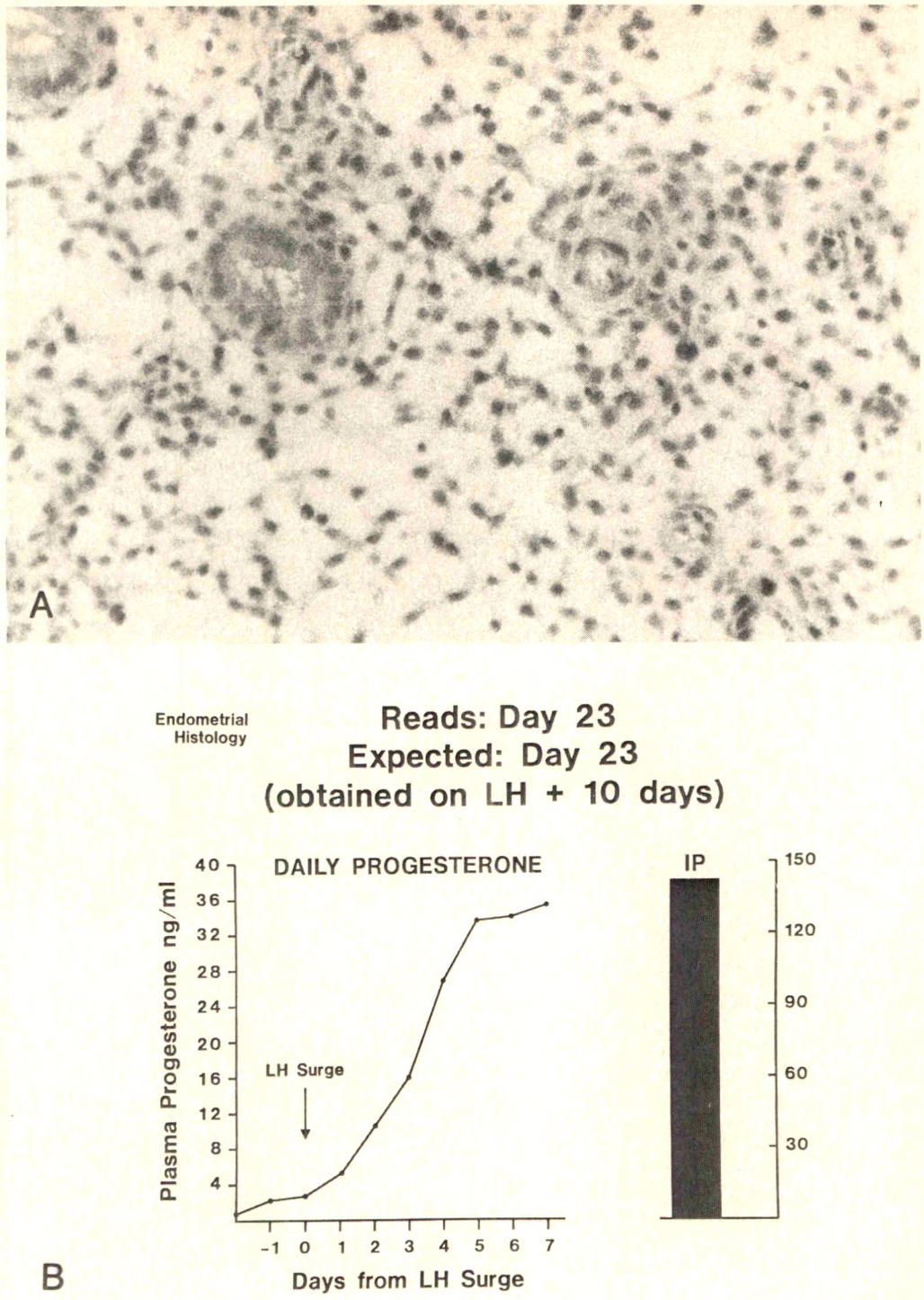


Fig. 2. Histologic appearance of in-phase endometrium **(A)** with corresponding integrated plasma progesterone value **(B)** for subject who received 150 mg clomiphene citrate daily for days 5 through 9 of cycle. Integrated plasma progesterone *(IP)* was calculated for 7 days after plasma LH surge.

intraobserver exact agreement less in the second half of the luteal phase than in the first half of the luteal phase.[12] We elected to perform the endometrial biopsies in the midluteal phase 7 to 10 days after the midcycle plasma LH surge and to specifically examine this window of time around implantation when histologic asynchrony or abnormalities would likely affect successful implantation. Furthermore, the optimum dose effect of progesterone should have been exerted on and the endometrium sufficiently transformed to be consistent



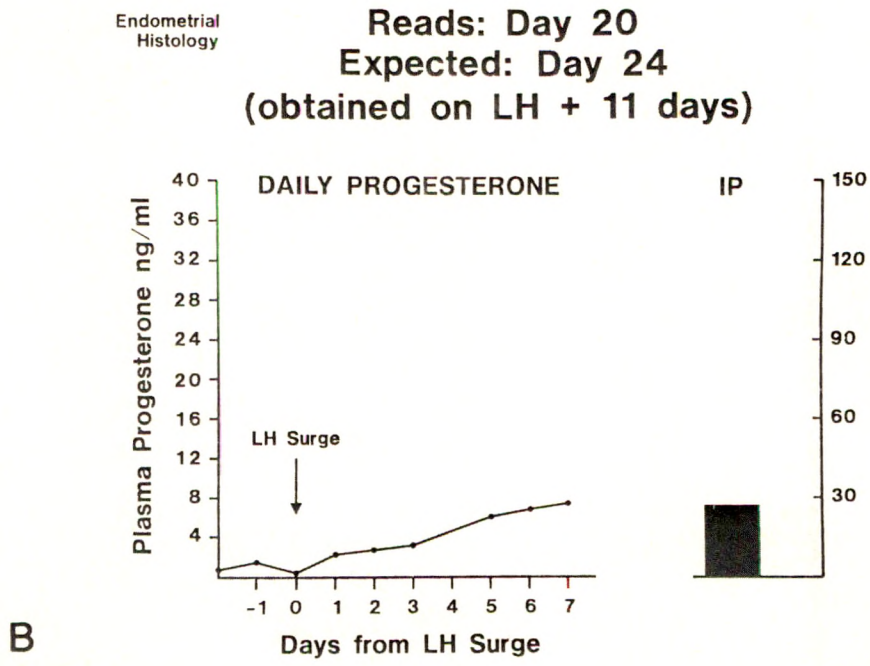


Fig. 3. Histologic appearance of out of phase endometrium **(A)** with the corresponding integrated plasma progesterone *(IP)* value **(B)** for subject who received 150 mg clomiphene citrate daily for days 5 through 9 of cycle. Integrated plasma progesterone *(IP)* was calculated for 7 days after plasma LH surge.

with preparation for successful implantation. Because of this consideration and also the slight variability in the chronologic timing after the plasma LH surge, we chose the integrated progesterone output on the basis of the first 7 days of the luteal phase after the midcycle LH surge. This would also be consistent with the time when implantation occurs. With use of the above criteria, we found that only 1 of 19 cycles (5%) treated

with clomiphene citrate produced a true luteal phase abnormality as a result of deficient progesterone. This cycle had a significantly reduced integrated progesterone output as well as an out-of-phase endometrium (delayed 4 days). The endometrial estrogen and progesterone receptors were normal,[5] and therefore the out-of-phase endometrium was a result of inadequate luteal progesterone output leading to deficient progesterone effect on the endometrium.

Failure to recognize the need to consider the cumulative effect of continuing luteal progesterone output on the endometrium and the wide variability in peripheral progesterone levels throughout the day, as well as throughout the luteal phase, has placed unnecessary emphasis on the significant correlation or lack thereof with peripheral progesterone levels. Area under the curve as reflected by integrated progesterone output calculated by the trapezoid method gives a closer reflection of the total amount of progesterone to which the endometrium has been exposed during the luteal phase up to that particular time and has been shown to be reliable in the assessment of luteal function.[15] Previous studies that found a high incidence or prevalence of luteal phase defects with clomiphene citrate treatment[16] were carried out in anovulatory or oligo-ovulatory women and used either endometrial dating with the criteria of Noyes et al.[3] or luteal phase single or up to three pooled or individual serum progesterone levels,[17] the pitfalls of which have been extensively mentioned.

From the present investigation with the use of chronologic endometrial dating with the midcycle plasma LH surge, area under the curve progesterone output, and data on endometrial estrogen and progesterone receptors,[5] we conclude that in ovulatory women, the incidence of luteal phase defects or inadequacy as a result of clomiphene citrate–induction of ovulation is low and probably is a result of inadequate luteal phase progesterone production.

REFERENCES

1. Jones GS, Maffezzoli RD, Strott CA, Ross GT, Kaplan G. Pathophysiology of reproductive failure after clomiphene-induced ovulation. Am J Obstet Gynecol 1970;108:847-67.
2. Garcia J, Jones GS, Wentz AC. The use of clomiphene citrate. Fertil Steril 1977;28:707-17.
3. Noyes RW, Hertig A, Rock JA. Dating the endometrial biopsy. Fertil Steril 1950;1:3-25.
4. Li T-C, Rogers AW, Lenton EA, Dockery P, Cooke I. A comparison between two methods of chronological dating of human endometrial biopsies during the luteal phase, and their correlation with histologic dating. Fertil Steril 1987;48:928-32.
5. Hecht BR, Khan-Dawood FS, Dawood MY. Periimplantation phase endometrial estrogen and progesterone receptors: effect of ovulation induction with clomiphene citrate. Am J Obstet Gynecol 1989;161:1688-93.
6. Hecht BR, Khan-Dawood FS, Dawood MY. The luteinizing hormone surge: timing and characteristics in the plasma and urine after clomiphene citrate treatment. Fertil Steril 1989;52:401-5.
7. Snowden EU, Khan-Dawood FS, Dawood MY. The effect of naloxone on endogenous opioid regulation of pituitary gonadotropins and prolactin during the menstrual cycle. J Clin Endocrinol Metab 1984;59:298-302.
8. Dawood MY. Circulating maternal serum progesterone in high-risk pregnancies. Am J Obstet Gynecol 1976;125:832-40.
9. Johannisson E, Parker RA, Landgren B-M, Diczfalusy E. Morphometric analysis of the human endometrium in relation to peripheral hormone levels. Fertil Steril 1982;38:564-71.
10. Lenton EA, Landgren B-M, Sexton L. Normal variation in the length of the luteal phase of the menstrual cycle: identification of the short luteal phase. Br J Obstet Gynaecol 1984;91:685-9.
11. Scott RT, Snyder RR, Strickland DM, et al. The effect of interobserver variation in dating endometrial histology on the diagnosis of luteal phase defects. Fertil Steril 1988;50:888-92.
12. Li T-C, Dockery P, Rogers AW, Cooke ID. How precise is histologic dating of endometrium using the standard dating criteria. Fertil Steril 1989;51:759-63.
13. Li T-C, Rogers AW, Dockery P, Lenton EA, Cooke ID. A new method of histologic dating of human endometrium in the luteal phase. Fertil Steril 1988;50:52-9.
14. Johannisson E, Landgren B-M, Rohr HP, Diczfalusy E. Endometrial morphology and peripheral hormone levels in women with regular menstrual cycles. Fertil Steril 1987;48:401-8.
15. Wu CH, Minassian SS. The integrated luteal progesterone: an assessment of luteal function. Fertil Steril 1987;48:937-40.
16. Keenan JA, Herbert CA, Bush JR, Wentz AC. Diagnosis and management of out-of-phase endometrial biopsies among patients receiving clomiphene citrate for ovulation induction. Fertil Steril 1989;51:964-7.
17. Abraham GE, Maroulis GB, Marshall JR. Evaluation of ovulation and corpus luteum function using measurements of plasma progesterone. Obstet Gynecol 1974;44:522-5.

Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis

Manubai Nagamani, MD, and Charles A. Stuart, MD

Galveston, Texas

Women with polycystic ovarian disease and hyperthecosis have hyperinsulinemia and insulin resistance. It is possible that insulin in supraphysiologic concentration exerts its steroidogenic action on ovarian stromal cells through insulin-like growth factor I receptors. We undertook this study to investigate whether the ovarian stroma of women with hyperthecosis has specific binding sites for insulin or insulin-like growth factor I. Ovarian stromal tissue was obtained from seven women with normal ovulatory cycles and from five women with hyperthecosis of the ovaries. Binding studies with insulin tagged with iodine 125 and insulin-like growth factor I tagged with iodine 125 revealed specific binding sites both for insulin and insulin-like growth factor I in the ovarian stroma. The binding of insulin tagged with iodine 125 in the ovarian stroma of women with hyperthecosis (3.4% ± 1.1% (±SE) per 100 μg protein) was significantly ($p < 0.04$) lower than that observed in normal premenopausal women (8.3% ± 1.6% per 100 μg protein). By contrast, the specific binding of insulin-like growth factor I tagged with iodine 125 in the ovarian stroma of women with hyperthecosis (7.1% ± 1.7% per 100 μg protein) was higher than that observed in the ovarian stroma from normal women (4.5% ± 1.7% per 100 μg protein), although the difference was not statistically significant. The affinity constants for these high-affinity receptors were similar (1.2 to 3.6 × 10^9 L/mol) in the two groups of women. These results indicate that (1) the ovarian stroma has specific binding sites both for insulin and for insulin-like growth factor I; (2) in women with hyperthecosis, the ovarian stroma has decreased binding sites for insulin but has normal concentrations of insulin-like growth factor I receptors; and (3) in women with hyperthecosis, stimulation of ovarian androgen synthesis by insulin may be mediated through the insulin-like growth factor I receptors. (Am J Obstet Gynecol 1990;163:1992-7.)

Key words: Insulin-like growth factor I receptors, insulin receptors, ovarian stroma, hyperthecosis, polycystic ovarian disease

Insulin resistance and hyperinsulinemia are often seen in association with ovarian hyperandrogenism.[1-3] A positive correlation between peripheral insulin levels and ovarian vein androgen levels has been observed in women with hyperandrogenism as a result of hyperthecosis of the ovaries.[4] In vitro studies indicate that insulin stimulates ovarian stromal androgen synthesis.[5] However, patients with hyperinsulinemia have extreme insulin resistance that is a result of either defective insulin receptors or a postreceptor defect.[6, 7] It is hard to explain how insulin exerts its steroidogenic action on the ovarian stroma in these women because its action in other target tissues is defective. It has been shown that insulin in high concentration is capable of binding to insulin-like growth factor I receptors.[8] It is possible that insulin, which is present in supraphysiologic concentrations in women with polycystic ovarian disease and hyperthecosis, exerts its steroidogenic action on the ovarian stromal cells through insulin-like growth factor I receptors.

We undertook this study to investigate whether normal ovarian stroma has specific binding sites for insulin-like growth factor I and insulin and whether the number of these receptors is increased or decreased in women with hyperthecosis.

Material and methods

Patients. We obtained ovarian stromal tissue from seven normal premenopausal women undergoing hysterectomy for nonendocrine problems (myoma uterus, endometriosis, and cervical dysplasia). All of them had normal ovulatory cycles and had no hirsutism. The mean age was 39 years (range, 30 to 46 years) and the mean body weight was 160 pounds (range, 126 to 198 pounds). The fasting glucose and insulin levels were normal. Ovarian stromal tissue was also obtained from

From the Department of Obstetrics and Gynecology and Department of Medicine, University of Texas Medical Branch.
Supported by National Institutes of Health Research Grant Nos. RO1-Ca 45181 and RO1-DK 33749 and a grant from the National Institutes of Health general clinical research centers (MO1-RR0073).
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint requests: Manubai Nagamani, MD, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77550.
6/6/24720

Table I. Clinical characteristics of women with hyperthecosis

Patient	*Age (yr)*	*% Ideal body weight*	*Hirsutism*	*Virilization*
A	38	160	Severe	Clitorimegaly
B	32	164	Severe	Clitorimegaly; temporal balding
C	30	200	Severe	Temporal balding
D	30	168	Severe	Clitorimegaly; temporal balding
E	38	163	Severe	Clitorimegaly

Table II. Hormonal studies in women with hyperthecosis

Patient	*Testosterone (ng/dl)*	*DHEAS (ng/ml)*	*LH/FSH (mIU/ml)*	*Fasting insulin (μU/ml)*	*Insulin response**
A	185	720	17/13	38	317
B	612	300	23/5	212	2634
C	138	700	13/8	52	685
D	249	627	4/10	42	2290
E	400	550	7/12	51	650
Normal range	20-90	500-3000	5-15	3-22	33-167

LH, Luteinizing hormone; *FSH,* follicle-stimulating hormone.
*Cumulative sum of serum insulin concentrations 1, 2, and 3 hours after oral administration of 75 gm glucose.

five virilized women with a long history of hirsutism who were undergoing laparotomy. Clinical characteristics and endocrine studies of these five women are summarized in Tables I and II. All these women were obese, had anovulatory cycles, and had been hirsute for many years. All of them were nulliparous and had failed to ovulate with clomiphene citrate (Clomid). None had acanthosis nigricans. The testosterone levels were high. The dehydroepiandrosterone (DHEA) sulfate levels were in the low-normal range. After a high-carbohydrate (300 gm) diet was observed for 3 days, a standard oral glucose tolerance test was performed in all subjects around 8 AM. Blood samples for glucose and insulin levels were obtained before and hourly for 3 hours after oral glucose (75 gm) administration. Fasting insulin levels and insulin response to oral glucose were elevated in all patients whereas the glucose levels were normal. All except patient D underwent hysterectomy with bilateral salpingo-oophorectomy for severe progressive hirsutism and virilization that did not respond to ovarian suppression with birth-control pills. A wedge biopsy of the ovaries was performed in patient D to rule out virilizing ovarian tumor because the testosterone levels were in the tumor range. In all patients, the ovaries were found to be enlarged to two to three times the normal size. Cut section of the ovaries revealed abundant ovarian stroma with few small follicular cysts in the cortex of the ovary. Histologic examination revealed extensive luteinization of the ovarian stroma in patients B, D, and E. Nests of luteinized cells were present in the ovarian stroma of patients A and C.

Binding studies with ovarian stroma. To prepare ovarian microsomal membranes, we used with minor modification a technique developed for cartilage plasma membranes.[9] Ovarian stromal tissue (400 to 500 mg) was homogenized in 15 ml 0.01 mol/L imidazole, 0.25 mol/L sucrose, and 0.005 mol/L ethylenediaminetetraacetic acid (EDTA), pH 7.5 (ISE buffer) at 0° C for 60 seconds at half-speed with a polytron (Brinkman Instruments, Westbury, N.Y.). The homogenate was centrifuged at 1000 *g* for 15 minutes to remove nuclei, most mitochondria, and tissue fragments. The resulting supernatant was centrifuged at 35,000 *g* for 60 minutes to provide a final pellet that was resuspended in 0.001 mol/L imidazol, pH 7.5.

Insulin and insulin-like growth factor I binding studies were performed in 0.1 mol/L HEPES acid, 0.120 mol/L sodium chloride, 0.0012 mol/L magnesium sulfate, 0.001 mol/L EDTA, 0.01 mol/L glucose, 0.015 mol/L sodium acetate, and 1% bovine serum albumin, pH 8.0, as previously described.[10] Studies designed for Scatchard analysis of binding parameters consisted of eight different concentrations of insulin performed in triplicate. The concentration of insulin tagged with iodine 125 was approximately 0.05×10^{-9} mol/L. Unlabeled porcine insulin was added to achieve concentrations of 0, 0.2, 0.5, 1, 2, 10, 20, and 1000×10^{-9} mol/L and the membrane protein concentration was ≤1 mg/ml. For insulin-like growth factor I binding studies, unlabeled insulin-like growth factor I and [^{125}I]insulin-like growth factor I were used in similar concentrations. The mixtures were incubated at 4° C for 15 to 18 hours and the membranes were sedimented by centrifugation at 18,000 *g* for 15 minutes and washed once in binding buffer. The results were expressed as the percentage of total radioactivity bound per 100 μg membrane protein. Protein was quantitated

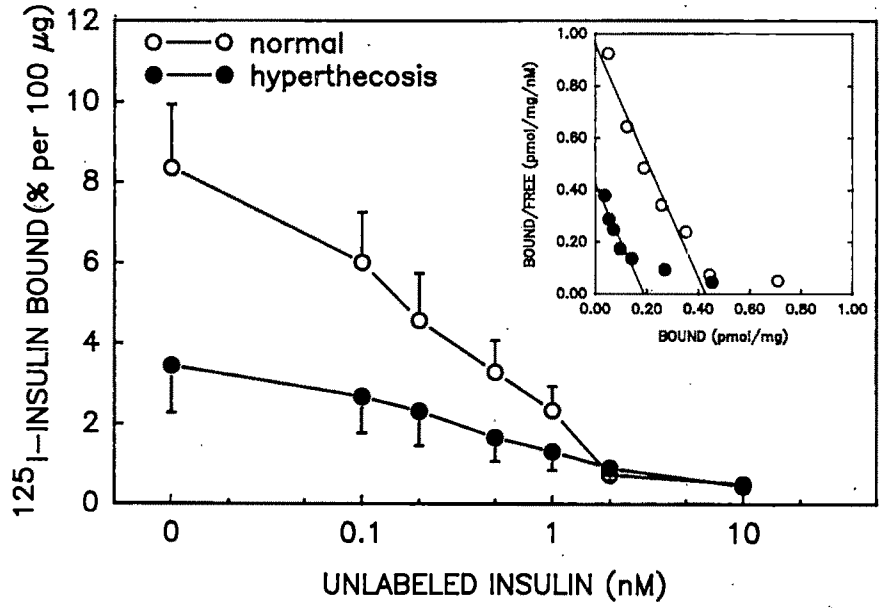


Fig. 1. Mean (±SE) specific insulin binding to ovarian stromal membranes from seven premenopausal women and five women with ovarian hyperthecosis. *Inset* shows Scatchard plot of same data.

by the method of Lowry et al.[11] Nonspecific binding was determined in the presence of at least 200-fold molar excess of unlabeled insulin and insulin-like growth factor I, respectively. Analysis of binding data was performed with a computer program designed to provide the best fit of a two-receptor model to the Scatchard plot. The program performs an iterative curve-stripping procedure in which a curvilinear Scatchard plot is recalculated stepwise, subtracting an increasing number of counts due to low-affinity specific binding until the optimal high-affinity straight line is achieved.[12] The independent Student t test was used to compare differences in specific binding between control women and women with hyperthecosis. A p value < 0.05 was considered significant.

Specificity studies. To evaluate the specificity of insulin-like growth factor I binding, ovarian stromal membranes were incubated in the presence of a constant amount of (1×10^5 counts per minute) of [^{125}I]insulin-like growth factor I, with or without increasing concentrations of unlabeled (1 to 100 ng/tube) insulin-like growth factor I or insulin (1 to 1000 ng/ml).

Results

Insulin binding studies. Our results indicate the presence of specific binding sites for insulin in normal ovarian stromal tissue. The percentage of total insulin binding in normal stroma was 8.3% ± 1.6% (±SE) per 100 μg protein and ranged from 4.3% to 15.2% per 100 μg protein. In women with hyperthecosis, the percent binding of [^{125}I]insulin (3.4% ± 1.1% ±SE) per 100 μg protein; range, 0% to 6.7% per 100 μg protein) was significantly lower ($p < 0.04$) than that observed in normal premenopausal women. The ovarian stroma of patient D did not have any specific binding sites for insulin. Fig. 1 shows the competition curves for binding of [^{125}I]insulin to ovarian stromal membranes of normal premenopausal women and women with hyperthecosis. The inset of Fig. 1 shows the mean data for Scatchard plots. The Scatchard plots were curvilinear, indicating either two binding sites of differing affinity or "negative cooperativity." The Scatchard plots of the high-affinity segments of the data (first five points) were parallel, indicating that the difference in insulin binding was a result of a difference in the concentration of the receptors rather than differences in affinity. The affinity constants for the specific high-affinity insulin receptors from these preparations were similar ($3.5 \pm 1.0 \times 10^9$ L/mol; normal ovarian stroma; and $3.5 \pm 0.8 \times 10^9$ L/mol/ hyperthecosis). The mean receptor concentration in the ovarian stroma of women with hyperthecosis (0.16 ± 0.07 pmol/mg membrane protein) was lower than that in the normal ovarian stroma (0.48 ± 0.18 pmol/mg membrane protein).

Insulin-like growth factor I binding studies. Specific binding sites for insulin-like growth factor I were present in the ovarian stroma from normal premenopausal women. The percentage of total binding of insulin-like growth factor I to normal ovarian stroma was 4.5% ± 1.7% per 100 μg protein (range, 2.0% to 7.4%/100 μg protein). The percentage of total binding insulin-like growth factor I in the ovarian stroma of women with hyperthecosis (7.1% ± 1.7% per 100 μg

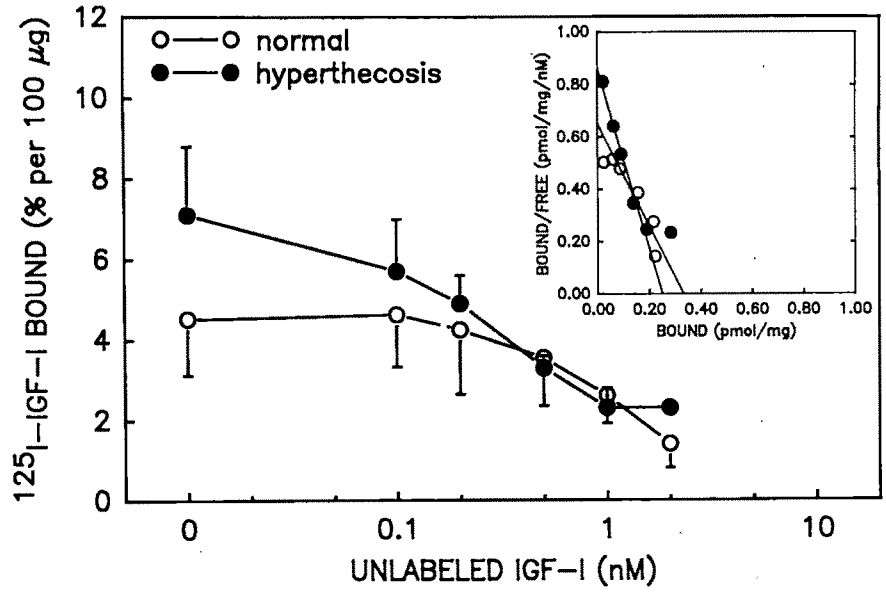


Fig. 2. Mean (± SE) specific insulin-like growth factor I *(IGF-I)* binding to ovarian stromal membranes from premenopausal women and women with hyperthecosis. *Inset* shows Scatchard plot of same data.

protein) was higher than that observed in normal ovarian stroma, but the difference was not statistically significant (Fig. 2). Specific binding sites for insulin-like growth factor I were present in all the ovaries with hyperthecosis (range, 4.1% to 12.7% per 100 μg protein). In patient D, the ovarian stroma did have binding sites for insulin-like growth factor I, even though there was no demonstrable insulin binding. The inset of Fig. 2 shows the mean data from Scatchard plots. The affinity constants for these insulin-like growth factor I receptors were 3.6 ± 1.5 × 10^9 L/mol in hyperthecosis and 1.2 ± 0.3 × 10^9 L/mol in the normal ovarian stroma. The difference was not statistically significant.

Specificity studies. Fig. 3 shows the competition curves for binding of [^{125}I]insulin-like growth factor I to ovarian stromal membranes prepared from normal ovarian stroma and women with hyperthescosis in the absence and presence of varying concentrations of unlabeled insulin-like growth factor I, insulin-like growth factor II, and insulin. Increasing concentrations (1 to 100 nmol/L) of unlabled insulin-like growth factor I resulted in dose-dependent saturable displacement of specific insulin-like growth factor I binding. Insulin was nonreactive at the final concentration of 1000 nmol/L and insulin-like growth factor II was only weakly effective. Results of our specificity studies indicate that human ovarian stromal cells possess separate receptors for insulin and insulin-like growth factor I.

Comment

Specific binding sites for insulin have been shown in the ovarian stroma of normal and polycystic ovaries.[13, 14] However, we are not aware of any previous reports that compare the receptor concentrations in the normal and polycystic ovaries. Our present data indicate that the specific binding sites for insulin are significantly decreased in the ovarian stroma of women with hyperthecosis. Lack of a difference in receptor affinity, as shown by the Scatchard plots, indicates that the reduced [^{125}I]insulin binding is primarily due to a reduction in the number of receptors. This decrease in insulin receptor concentrations could be a result of either a primary receptor defect or a down regulation in response to elevated insulin levels. Poretskey et al.[15] observed a 40% decrease in insulin binding in ovarian tissue when the concentration of insulin in the preincubation medium was 10 ng/ml and complete obliteration of [^{125}I]insulin binding when the concentration was >50 ng/ml. Maximal plasma insulin concentration of 300 to 1000 μU/ml that we observed in our patients after oral glucose ingestion is similar to the dosages used in this study (50 ng/ml ~ 1250 μU/ml).

In the present study, all our patients with hyperthecosis had hyperinsulinemia with normal glucose levels, a finding that indicates insulin resistance and defective insulin action on carbohydrate metabolism. The ability of insulin to alter ovarian function in the face of insulin resistance could be due to point mutations in the insulin receptor that lead to an inability of the insulin receptor to modulate glucose metabolism but do not affect the ability of insulin to bind to the receptor and modulate other functions such as steroidogenesis. The action of hyperinsulinemia on ovarian androgen production may be a result of direct effects of insulin on its own

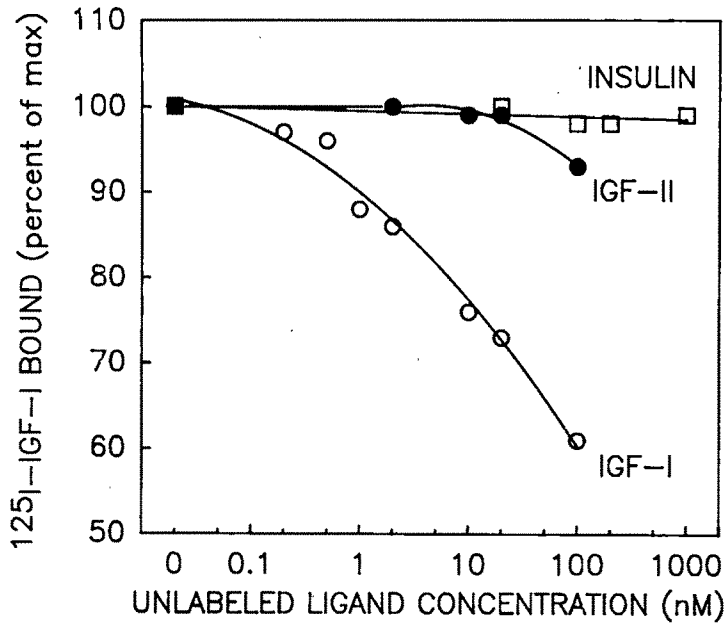


Fig. 3. Specificity of insulin-like growth factor I *(IGF-I)* binding to ovarian stromal membranes.

receptor or cross-over stimulation via the insulin-like growth factor I receptor. Our specificity data shown in Fig. 3 do not support crossover action in this subject because insulin concentrations as high as 1000 nmol/L did not displace [^{125}I]insulin-like growth factor I. However, in many tissues the relative potency of insulin in the prevention of binding of insulin-like growth factor I is 1/50 to 1/200, suggesting that hyperinsulinemia could result in activation of the insulin-like growth factor I receptor.[8] This supposition is further supported by the fact that patients with type B severe insulin resistance, who have antibodies to insulin receptors, have ovarian hyperandrogenism.[16]

Insulin-like growth factor I receptors have previously been shown to be present in the rat interstitial and granulosa cells and swine granulosa cells.[17-19] Specific binding sites for insulin-like growth factor I have also been shown to be present on human granulosa cells.[20] We showed for the first time that there are insulin-like growth factor I receptors in the normal human ovarian stroma. Unlike insulin receptors, the mean receptor concentration of insulin-like growth factor I in ovarian stroma of women with hyperthecosis was higher than that in normal ovarian stroma, even though the difference did not achieve statistical significance. It has been shown in the rat that experimetnal hyperinsulinemia upregulates ovarian insulin-like growth factor I receptors.[21] Small differences in the receptor concentration between the stroma from normal ovary and that from the polycystic ovary could also be a result of the differences in the cell type. Studies by Erikson et al.[22] indicate that the ovarian stromal tissue is heterogeneous and contains primary interstitial cells that have little or no capacity to synthesize steroids and secondary interstitial cells that secrete androgens. In the ovarian stroma of polycystic ovaries, there is a marked increase in the number of steroidogenically active secondary interstitial cells. It is possible that these secondary interstitial cells have an increased number of binding sites for insulin-like growth factor I and the stimulation of androgen synthesis by insulin is mediated through insulin-like growth factor I receptors. The only previous attempt to identify insulin-like growth factor I receptors in the human ovarian stroma is that of Poretsky and colleagues.[14] In that study, minced ovarian tissue rather than stromal membrane preparation was incubated with [^{125}I]insulin-like growth factor I, and the failure to identify insulin-like growth factor I receptors could be because of the difference in methods. After modification of the assay conditions, these authors were able to show specific, high-affinity insulin-like growth factor I binding sites on human granulosa cells.[20]

Inasmuch as the human ovary has both insulin-like growth factor I and insulin receptors, insulin could mediate its action on ovarian steroidogenesis either through its own receptor or through insulin-like growth factor I receptors. In the normal ovary under physiologic conditions, insulin may exert its effect mainly through its own receptors, whereas in polycystic ovarian disease and hyperthecosis, when the insulin receptors may be defective and insulin is present in pharmacologic concentrations, insulin might mediate its action through insulin-like growth factor I receptors. This is further supported by the fact that the ovarian

stroma of patient D, who had severe hyperinsulinemia and ovarian hyperandrogenism, had no measurable insulin receptors.

In conclusion, our present data indicate that (1) the ovarian stroma has specific binding sites both for insulin and for insulin-like growth factor I; (2) in women with hyperthecosis, the ovarian stroma has decreased binding sites for insulin, but has normal or increased concentration of insulin-like growth factor I receptors; and (3) in women with hyperthecosis and hyperinsulinemia, stimulation of ovarian androgen synthesis by insulin may be mediated through the insulin-like growth factor I receptors.

REFERENCES

1. Chang RJ, Nakamura RM, Judd HL, Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metab 1983;57:356-9.
2. Shoupe D, Kumar DD, Lobo RA. Insulin resistance in polycystic ovary syndrome. AM J OBSTET GYNECOL 1983; 147:588-92.
3. Burgen GA, Given JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50:113-6.
4. Nagamani M, Dinh TV, Kelver ME. Hyperinsulinemia in hyperthecosis of the ovaries. AM J OBSTET GYNECOL 1986;154:384-9.
5. Barbieri RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. Insulin stimulates androgen accumulation in incubation of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab 1986; 62:904-10.
6. Podskalny JM, Kahn CR. Cell culture studies on patients with extreme insulin resistance I receptor defects on cultured fibroblasts. J Clin Endocrinol Metab 1982;54: 261-8.
7. Bar RS, Muggeo M, Roth J, Kahn CR, Kavrankova J, Imperato-McGinley J. Insulin resistance, acanthosis Nigricans, and normal insulin receptors in a young woman: evidence for a post receptor defect. J Clin Endocrinol Metab 1978;47:620-5.
8. Rechler MM. The nature and regulation of the receptors for insulin-like growth factors. Annu Rev Physiol 1985; 47:425-42.
9. Stuart CA, Furlanetto RW, Lebovitz HE. The insulin receptor of embryonic chick cartilage. Endocrinology 1979; 105:1293-302.
10. Nagamani M, Hannigan EV, Dinh TV, Stuart CA. Hyperinsulinemia and stromal luteinization of the ovaries in postmenopausal women with endometrial cancer. J Clin Endocrinol Metab 1988;67:144-8.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:267.
12. Stuart CA. Phylogenetic distance form mean correlates with immunologic cross-reactivity among liver insulin receptors. Comp Biochem Physiol 1986;84B:167-72.
13. Poretsky L, Smith D, Seibel M, Pazianos M, Moses AC, Flier JS. Specific insulin binding sites in human ovary. J Clin Endocrinol Metab 1984;59(4):809-11.
14. Poretsky L, Grigorescu F, Seibel M, Moses AC, Flier JS. Distribution and characterization of insulin and insulin-like growth factor I receptors in normal human ovary. J Clin Endocrinol Metab 1985;61:728-34.
15. Poretsky L, Bhargava G, Kalin MF, Wolf SA. Regulation of insulin receptors in the human ovary: in vitro studies. J Clin Endocrinol Metab 1988;67:774-8.
16. Taylor SI, Dons RF, Hernandez E, Roth J, Gordon P. Insulin resistance associated with androgen excess in women with auto antibodies to the insulin receptor. Ann Intern Med 1982;97:851-5.
17. Hernandez RE, Resnick CE, Svolboda EM, VanWyk JJ. Somatomedin-C/insulin-like growth factor I as an enhancer of androgen biosynthesis by cultured rat ovarian cells. Endocrinology 1988;122:1603-12.
18. Adashi EY, Resnick EC, Hernandez ER, Svoboda ME, VanWyk JJ. Characterization and regulation of a specific cell membrane receptor for somatomedin-C/insulin-like growth factor I in cultured rat granulosa cells. Endocrinology 1988;122:194-201.
19. Veldhuis JD, Furlanetto RW. Tropic action of human somatomedin C/insulin-like growth factor I on ovarian cells: in vitro studies with swine granulosa cells. Endocrinology 1985;116:1235-42.
20. Gates GS, Bayer S, Seibel M, Poretsky L, Flier SJ, Moses AC. Characterization of insulin-like growth factor binding to human granulosa cells obtained during in vitro fertilization. J Recept Res 1987;7:885-902.
21. Poretsky L, Glover B, Laumas V, Kalin M, Dunaif A. The effects of experimental hyperinsulinemia on steroid secretion, ovarian [^{125}I]insulin binding, and ovarian [^{125}I]insulin-like growth factor I binding in the rat. Endocrinology 1988;122:581-5.
22. Erickson GF, Magoffin DA, Dyer CA, Hofeditz C. The ovarian androgen producing cells: a review of structure function relationship. Endocrinol Rev 1985;6:371-99.

Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women

Robert A. Wild, MD,[a] Mary Bartholomew, MS,[b] Deborah Applebaum-Bowden, PhD,[c] Laurence M. Demers, PhD,[d,e] William Hazzard, MD,[c] and Richard J. Santen, MD[d]

Oklahoma City, Oklahoma, Hershey, Pennsylvania, and Winston-Salem, North Carolina

Fifty-one hyperandrogenic women had their lipoprotein lipid profiles determined. Free and albumin-bound testosterone was associated with triglycerides and with high-density lipoprotein cholesterol independent of fasting insulin levels, percent ideal body weight, and waist/hip ratio. To gain insight into mechanisms of these lipid alterations, the women were subgrouped according to apparent source of androgen excess. Whereas all groups had low levels of high-density lipoprotein-2 cholesterol and high triglyceride concentrations, only in those with high luteinizing hormone–to–follicle-stimulating hormone ratios was free and albumin-bound testosterone associated with triglycerides and high-density lipoprotein cholesterol independent of fasting insulin levels. Relationships between percent ideal body weight and waist/hip ratios, free and albumin-bound testosterone, sex hormone binding globulin, fasting insulin and 2-hour insulin levels and blood pressure are not significant in all subgroups, suggesting differing endocrinological influences and differing mechanisms for lipoprotein lipid alterations. (AM J OBSTET GYNECOL 1990;163:1998-2005.)

Key words: Hyperandrogenism, lipoprotein lipids, polycystic ovaries, hirsutism, coronary vascular disease

We previously found that women with polycystic ovary syndrome have higher triglyceride levels and lower values of high-density lipoprotein (HDL) cholesterol concentrations than those of regularly menstruating nonhirsute, nonhyperandrogenic women.[1] These alterations are apparent when women with polycystic ovary syndrome are compared with normal women matched for body weight.[2] We found that in a separate group of hirsute hyperandrogenic women these lipoprotein lipid alterations are independently associated with elevated levels of insulin and androgen.[3]

There are a number of altered endocrinological influences in hirsute women that can impact on lipid metabolism. Because hirsute women are heterogeneous with respect to sources of androgen excess, we subgrouped our patients according to apparent source of androgen excess to gain insight into possible mechanisms for these lipoprotein lipid alterations.

From the Department of Obstetrics and Gynecology, University of Oklahoma Health Sciences Center,[a] Center for Biostatistics, Hershey Medical Center,[b] Department of Medicine, Bowman Gray School of Medicine,[c] Department of Medicine, Hershey Medical Center,[d] and Department of Pathology, Hershey Medical Center.[e]
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Supported in whole or in part by grants from Pennsylvania American Heart Association, Grant No. S07-RR 05680-19 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health, and the Presbyterian Health Foundation of Oklahoma City, Oklahoma.
Reprint requests: Robert A. Wild, MD, Oklahoma University Health Sciences Center, Department of Obstetrics and Gynecology, 920 S.L. Young Blvd., Oklahoma City, OK 73104.
6/6/24756

Material and methods

Fifty-one women with hirsutism were evaluated by history, physical examination, determination of androgens, estrogens, gonadotropins, and fasting and 2-hour postprandial glucose and insulin concentrations. Patients were sampled in the proliferative nonperiovulatory phase of their cycles. Assays were performed in the Core Endocrine Laboratory of the Hershey Medical Center by well-established previously reported procedures.[3] Lipoprotein lipids were determined in the Lipid Laboratory, Department of Medicine, Bowman Gray School of Medicine.[3] All assays were standardized in accordance with the Center for Disease Control. Fourteen-hour fasting lipoprotein lipid profiles were obtained from persons known not to have consumed alcohol for 3 days before sampling. A portion of these results have been reported previously.[3]

Patients with Cushing syndrome, androgen-secreting tumors, or prolactinomas were excluded. All women were in excellent general health. For 3 months before the study, none had taken any medications known to affect pituitary-gonadal function, blood pressure, carbohydrate or lipid metabolism. None had blood pressure >140 mm Hg systolic or 90 mm Hg diastolic. Two women were smokers (5 half pack years and 13 one-pack years) and two were prior smokers (5 years before, but less than a half pack per day for 3 years in their lifetimes). None consumed excessive amounts of alcohol, and none was vegetarian or eating any other special diet. Dietary history, exercise frequency, menstrual history, and family history of cardiovascular disease were

Table I. Correlations, partial correlations, and p values between lipoprotein lipids free and albumin-bound testosterone lipoprotein lipids and sex hormone binding globulin in women with hirsutism ($N = 51$)

		Partial adjusting			
	Correlation	*For fasting insulin*	*For fasting insulin, % IBW*	*For fasting insulin, waist/hip*	*For all 3*
Triglycerides and free and albumin-bound testosterone	0.42 (<0.01)	0.56 (<0.01)	0.60 (<0.01)	0.54 (<0.01)	0.58 (<0.01)
HDL cholesterol and free and albumin-bound testosterone	−0.35 (0.02)	−0.33 (0.04)	−0.32 (0.04)	−0.30 (0.06)	−0.30 (0.06)
Triglycerides and sex hormone binding globulin	−0.46 (<0.01)	−0.35 (0.03)	−0.42 (<0.01)	−0.33 (0.04)	−0.43 (<0.01)
HDL cholesterol and sex hormone binding globulin	0.50 (<0.01)	0.43 (<0.01)	0.45 (<0.01)	0.42 (<0.01)	0.45 (<0.01)

uT, Free and albumin-bound testosterone; *SHBG,* sex hormone binding globulin; *IBW,* ideal body weight.

recorded and scored for each patient as described previously.[1]

Corticotropin-stimulation studies to test for adult-onset congenital adrenal hyperplasia were not performed because of the infrequence of its occurrence outside of high-risk populations.[4] None of the women had a clinical history that suggested congenital adrenal hyperplasia (i.e., signs of virilization, early onset of symptoms, short stature, appropriate family history, or Ashkenazi Jewish or Eskimo ancestry.

All women had documented hyperandrogenism. To gain insight into pathophysiologic mechanisms responsible for lipoprotein lipid alterations the women were evaluated and subgrouped according to apparent source of androgen excess. Classic polycystic ovary syndrome includes luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio >1.5 and elevations of one or more androgens, free and albumin-bound testosterone, androstenedione, or dehydroepiandrosterone sulfate (DHEAS). Idiopathic ovarian hyperandrogenism includes women with LH/FSH ratio <1.5 and no elevation of DHEAS level. Idiopathic adrenal hyperandrogenism includes persons with elevated DHEAS levels with LH/FSH ratio <1.5 with or without elevated androstenedione concentrations. High-density lipoprotein-2 (HDL_2) cholesterol measurements were obtained in nine patients with polycystic ovary syndrome, in 12 patients with idiopathic ovarian hyperandrogenism, and in four patients with idiopathic adrenal hyperandrogenism. Pearson correlations were made between the lipoprotein lipid parameters, the androgen, insulin, and blood pressure parameters, percent ideal body weight, and waist/hip ratios in each subgroup. Analysis of variance was used to compare mean concentrations of parameters of interest in the three subgroups and to test for differences in mean levels of lipids, androgens, and insulin variables according to waist/hip categories <0.75, >0.75, <0.85, and >0.85 for the entire group of hyperandrogenic women.[5] For the entire group, partial correlation analysis was used to determine independent association of free and albumin-bound testosterone and sex hormone binding globulin with HDL cholesterol and triglycerides independent of fasting insulin levels, percent ideal body weight, and waist/hip ratio. The associations of fasting insulin and 2-hour insulin values with HDL cholesterol and triglycerides independent of free and albumin-bound testosterone, percent ideal body weight, and waist/hip ratio were also described by partial correlation analysis. Partial correlation was also used to determine independent associations of free and albumin-bound testosterone with triglycerides and with HDL cholesterol independent of fasting insulin and 2-hour insulin levels in the women with polycystic ovary syndrome. Log transformations of triglyceride and insulin concentrations were made before analysis.

Results

The correlations and partial correlation coefficients between HDL cholesterol and triglycerides and free and albumin-bound testosterone and sex hormone binding globulin for the entire group of hirsute women are shown in Table I. After adjustment for potential interactions, we found significant associations suggesting that androgen has an influence on lipoprotein lipid profiles independent of insulin levels, percent ideal body weight, and waist/hip ratios.

In contrast, the converse associations of fasting insulin and 2-hour insulin levels (complete data set not shown) with triglycerides ($r = 0.52, p < 0.01$) and HDL cholesterol ($r = -0.39, p < 0.01$) differed when corrected for free and albumin-bound testosterone, percent ideal body weight, and waist/hip ratio. Triglycerides were associated with fasting insulin and 2-hour

Table II. Clinical parameters and lipoprotein lipids, insulins, and androgens in hirsute women ($N = 51$)

	Units	*PCOS**	*IOH**	*IAH**
Age	Years	29 ± 1.4	31 ± 1.3	27 ± 2.3
Weight	Pounds	180 ± 8.4	186 ± 8.6	202 ± 15.8
% IBW		134 ± 6.2	140 ± 6.0	152 ± 12.5
LH/FSH†	mIU/mol	2.3 ± 0.14	0.8 ± 0.07	0.9 ± 0.13
Testosterone‡	ng/dl	116 ± 12.3	84 ± 7.8	126 ± 12.5
Free and albumin-bound testosterone	ng/dl	62 ± 10.8	40 ± 4.9	61 ± 5.6
SHBG	nmol/L	21 ± 2.4	22 ± 1.8	17 ± 2.8
Androstenedione	ng/ml	3.0 ± 0.31	2.3 ± 0.22	3.3 ± 0.41
DHEAS§	ng/ml	2633 ± 481	2005 ± 130	5155 ± 525
Triglycerides	ng/dl	154 ± 36.4	128 ± 12.6	130 ± 31.5
HDL cholesterol	ng/dl	44 ± 3.8	45 ± 2.9	42 ± 3.8
HDL_2	ng/dl	0.76	1.2	3.3
Fasting insulin	uU/ml	22 ± 4.9	22 ± 4.5	44 ± 26.3
2-hour insulin	uU/ml	176 ± 64.5	96 ± 20.6	90 ± 34.0

PCOS, Polycystic ovary syndrome; *IOH,* idiopathic ovarian hyperandrogenism; *IAH,* idiopathic adrenal hyperandrogenism; IBW, ideal body weight; *SHBG,* sex hormone binding globulin.

*Mean hormone concentrations ± SEM. Nine patients with polycystic ovary syndrome, 12 with idiopathic ovarian hyperandrogenism, and four with idiopathic adrenal hyperandrogenism had HDL_2 measured.

†Polycystic ovary syndrome greater than others.

‡Idiopathic adrenal hyperandrogenism greater than idiopathic ovarian hyperandrogenism.

§Idiopathic adrenal hyperandrogenism greater than others.

Table III. Potential confounding variables in 51 hyperandrogenic women

	No. reporting being regular	*Alcohol 1-2 times/wk*	*No exercise*	*Close positive family history*
PCO	3/17	3/19	8/17	4/14
IOH	6/25	2/25	8/26	11/25
IAH	5/8	0/8	5/8	4/8

PCO, Polycystic ovary syndrome; *IOH,* idiopathic ovarian hyperandrogenism; *IAH,* idiopathic adrenal hyperandrogenism.

insulin levels ($r = 0.37$, and $r = 0.54$, respectively, $p < 0.01$) after correcting for potential interactions with free and albumin-bound testosterone, percent ideal body weight, and waist/hip ratio. However, the associations of HDL cholesterol and fasting insulin and 2-hour insulin levels became nonsignificant when corrections were made for potential interactions.

For the entire group, persons with waist/hip ratios >0.85 had higher fasting and 2-hour postprandial insulin concentrations than those with waist/hip ratios <0.85. Concentrations of sex hormone binding globulin were higher in women with waist/hip ratios <0.75 ($p < 0.05$).

The data suggest that androgen may have more of an effect on HDL cholesterol concentrations and insulin may alter triglyceride metabolism. However, both androgens and insulin may alter lipoprotein lipid metabolism in hirsute women.

The androgen, lipoprotein lipid, and insulin parameters, along with mean ages, weights, and percent ideal body weight for the subgroups of women with hyperandrogenism are shown in Table II. Significant differences among average levels in the three groups were not seen except in LH/FSH ratios, testosterone concentrations, and DHEAS concentrations. Average values for weight, percent ideal body weight, waist/hip ratio, systolic or diastolic blood pressure were not different. The women with idiopathic adrenal hyperandrogenism were heavier but not statistically so. Other potential confounding variables as they related to the three subgroups of the hyperandrogenic women are shown in Table III. None was identified as being significant.

In women with either idiopathic ovarian or idiopathic adrenal hyperandrogenism, no association of any of the lipoprotein lipids was found with any of the androgens or the insulin parameters. The small numbers of patients in the group with idiopathic adrenal hyperandrogenism prevent definitive conclusions; however, all groups were found to have elevated triglyceride levels and all groups were found to have low concentrations of HDL cholesterol. Evidence of insulin resistance was

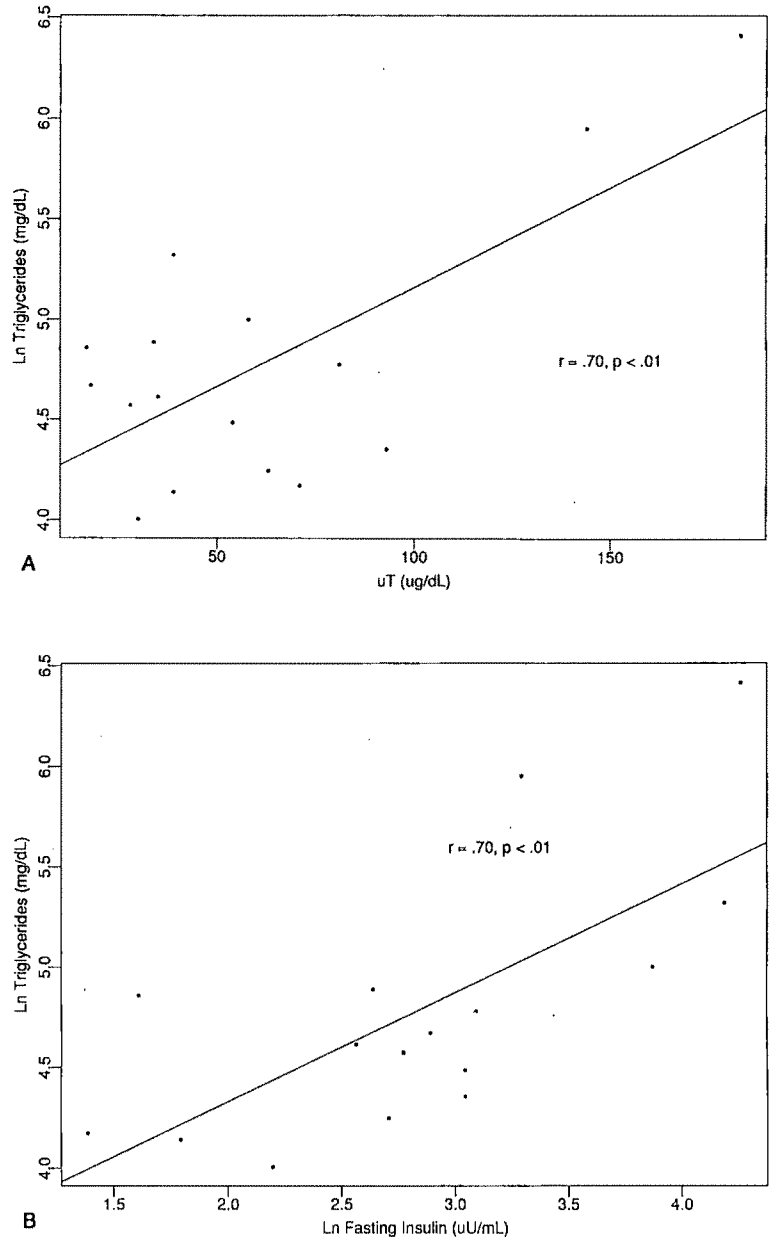


Fig. 1. Association of triglycerides with free and albumin-bound testosterone *(uT)*, **A,** and fasting insulin, **B,** in women with polycystic ovary syndrome.

found in polycystic ovary syndrome and idiopathic adrenal hyperandrogenism subgroups as well, as noted by abnormal high fasting insulin and 2-hour insulin concentrations. All groups had low levels of HDL_2 cholesterol.

Only in women with polycystic ovary syndrome were both free and albumin-bound testosterone (Figs. 1, *A* and 2, *A*) and fasting insulin (Figs. 1, *B* and 2, *B*) associated with triglycerides and negatively with HDL cholesterol (Fig. 2). Note that after adjustment for the effect of insulin, free and albumin-bound testosterone is significantly associated with triglyceride (Fig. 3, *A*) and HDL cholesterol concentrations in these women (Fig. 3, *B*). Fasting insulin level was not associated with HDL cholesterol independent of the effect of free and albumin-bound testosterone.

Tables III and IV show the association of percent ideal body weight and waist/hip ratios to the parameters of interest for the entire group and for the three different subsets.

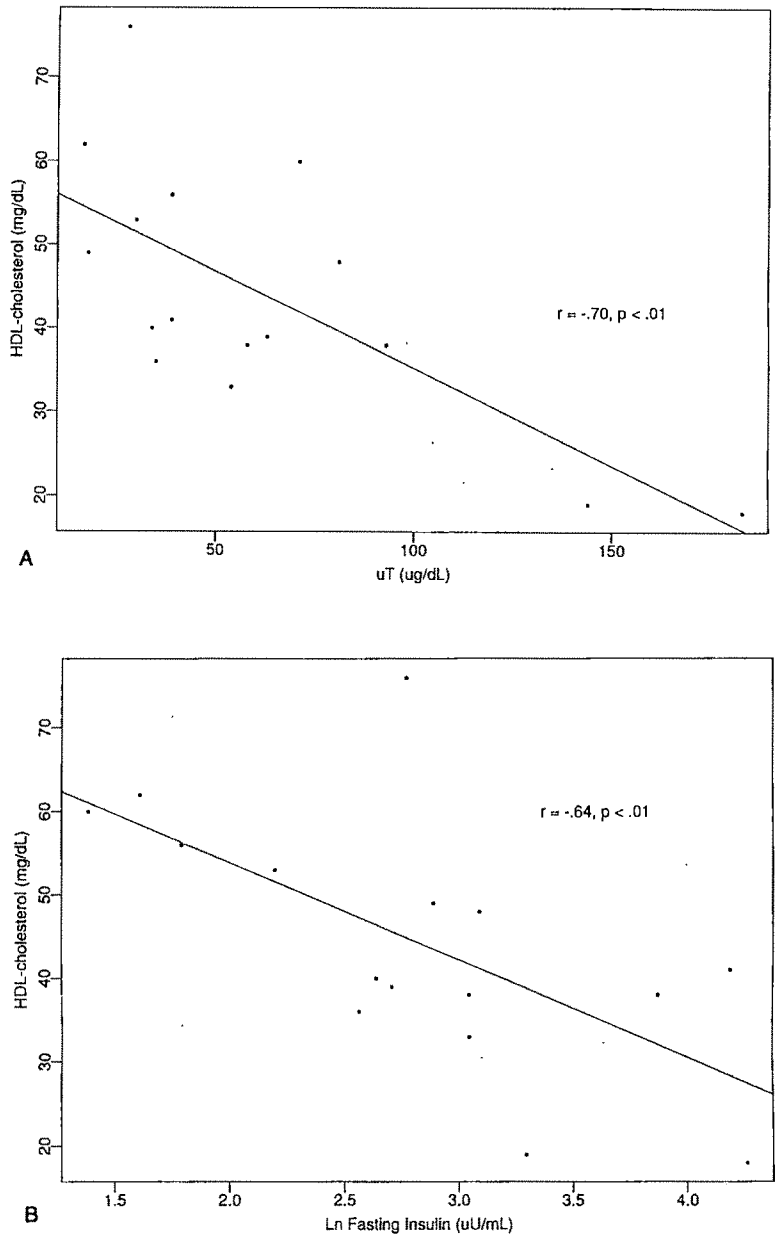


Fig. 2. Association of HDL cholesterol with free and albumin-bound testosterone *(uT)*, **A,** and fasting insulin, **B,** in women with polycystic ovary syndrome.

The associations of free and albumin-bound testosterone and HDL cholesterol were significant for the entire group but not for idiopathic ovarian hyperandrogenism and idiopathic adrenal hyperandrogenism. The strength of the association of free and albumin-bound testosterone with triglycerides and HDL cholesterol in the entire group is therefore carried by the associations found in women with high LH/FSH ratios, depicted here as those with polycystic ovary syndrome.

Comment

Women with hyperandrogenism are heterogeneous with respect to sources of androgen excess. With the exception of a few well-defined inherited disorders[6] any classification of them is arbitrary because there is no uniform agreement as to what constitutes essential criteria for classification. Because LH stimulates ovarian androgen production we have classified our women with androgen excess into those with polycystic ovary syndrome, those with idiopathic ovarian hyperandrogenism, and those with idiopathic adrenal hyperandrogenism to gain insight into possible mechanisms for their demonstrated lipoprotein lipid alterations. Whereas LH is pulsatile, and whereas its pulsatility makes a large difference for any given patient or for small numbers of patients, with pooled group data this

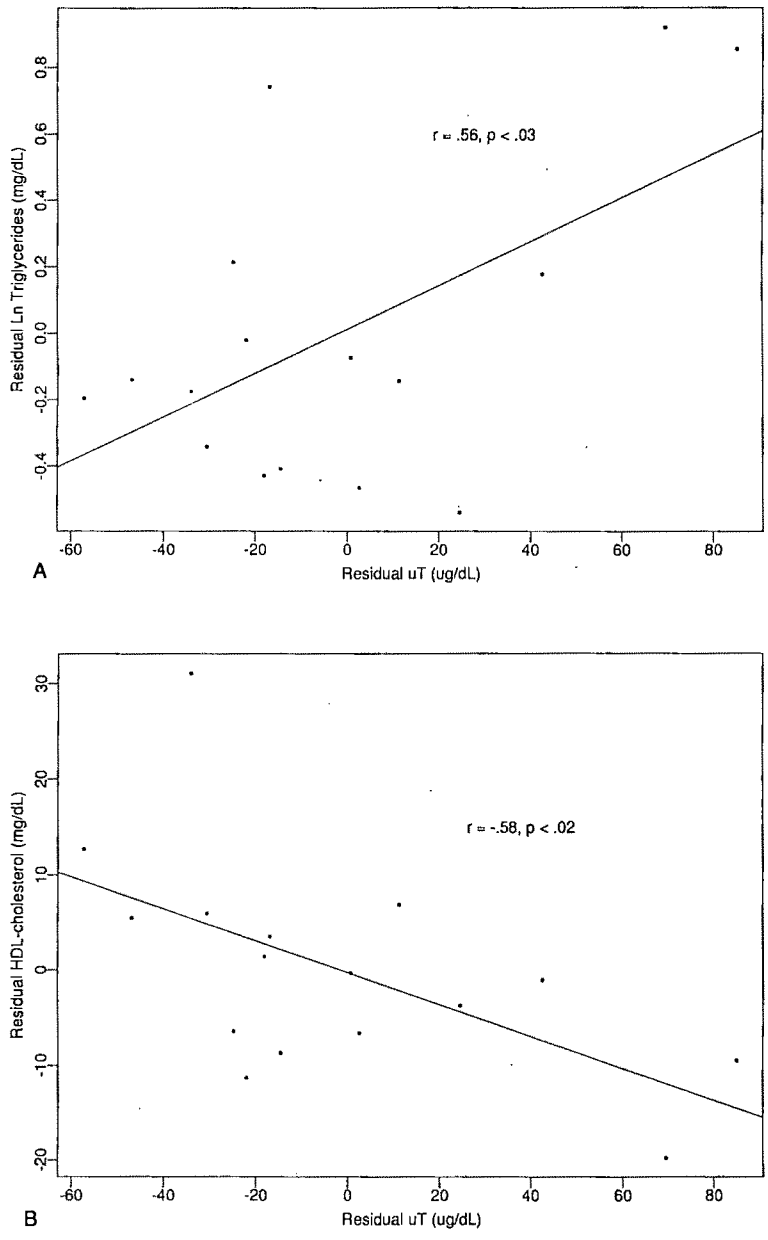


Fig. 3. Association of triglycerides **A**, and HDL cholesterol, **B**, with free and albumin-bound testosterone *(uT)* independent of insulin in women with polycystic ovary syndrome.

makes little difference.[7] It is recognized that the terms *polycystic ovary syndrome, idiopathic adrenal hyperandrogenism,* and *idiopathic ovarian hyperandrogenism* are arbitrary because of potential mixed sources of hyperandrogenism. Even though all women had low HDL and HDL_2 cholesterol levels and elevated triglyceride levels, only those women with high LH values were found to have the previously described associations of free and albumin-bound testosterone and fasting insulin with triglycerides and HDL cholesterol. As previously found[3] free and albumin-bound testosterone was associated positively with triglycerides and negatively with HDL cholesterol independent of insulin.

It is possible that the well-described insulin resistance[8] and the higher amounts of LH-dependent androgen excess in polycystic ovary syndrome influence lipoprotein lipid metabolism. The role of insulin excess associated with insulin resistance in women with polycystic ovary syndrome has had much recent attention. Insulin may act on the ovary either through its own receptor or through insulin-like growth factor I receptors to contribute to androgen production, which may secondarily affect lipoprotein lipid metabolism in these women. Insulin is known to potentiate the effect of LH on ovarian stromal androgen production.[9] Specific binding sites for insulin-like growth factor I in the ovar-

Table IV. Correlations of percentage of ideal body weight to lipids, insulins, and androgens in hyperandrogenic women

Variable	*Entire group (N = 51)*	*PCOS (N = 17)*	*IOH (N = 26)*	*IAH (N = 8)*
Cholesterol	0.20 (0.17)	0.30 (0.26)	0.55 (<0.01)	−0.13 (0.79)
Triglycerides	0.14 (0.36)	−0.12 (0.67)	0.34 (0.10)	0.07 (0.87)
HDL cholesterol	−0.17 (0.25)	−0.22 (0.40)	−0.08 (0.72)	−0.40 (0.37)
Free and albumin-bound testosterone	0.22 (0.13)	0.01 (0.96)	0.50 (0.01)	0.44 (0.27)
SHBG	−0.43 (<0.01)	−0.25 (0.34)	−0.57 (<0.01)	−0.39 (0.39)
Fasting insulin	0.43 (<0.01)	0.50 (<0.04)	0.49 (0.01)	0.12 (0.79)
2-hour insulin	0.35 (0.01)	0.33 (0.19)	0.42 (0.03)	0.47 (0.24)
Systolic BP	0.32 (0.02)	0.06 (0.82)	0.58 (<0.01)	−0.24 (0.56)
Diastolic BP	0.27 (0.06)	0.03 (0.91)	0.58 (<0.01)	−0.31 (0.45)

PCOS, Polycystic ovary syndrome; *IOH*, idiopathic ovarian hyperandrogenism; *IAH*, idiopathic adrenal hyperandrogenism; *SHBG*, sex hormone binding globulin; *BP*, blood pressure. All *p* values are in parentheses.

Table V. Correlation of waist/hip ratio to lipids, insulins, and androgens in hirsute women

Variable	*Entire group N = 51*	*PCOS N = 17*	*IOH N = 26*	*IAH N = 8*
Cholesterol	0.09 (0.53)	−0.03 (0.92)	0.10 (0.62)	−0.34 (0.45)
Triglycerides	0.39 (<0.01)	−0.40 (0.12)	0.35 (0.09)	0.58 (0.17)
HDL cholesterol	−0.30 (0.04)	−0.33 (0.22)	−0.27 (0.22)	−0.38 (0.40)
Free and albumin-bound testosterone	0.23 (0.11)	−0.25 (0.33)	0.23 (<0.26)	0.58 (0.13)
SHBG	−0.27 (0.07)	−0.07 (0.78)	−0.37 (<0.10)	−0.65 (0.11)
Fasting insulin	0.46 (<0.01)	0.72 (0.01)	0.32 (<0.11)	0.26 (0.57)
2-hour insulin	0.38 (<0.01)	0.58 (0.01)	0.24 (0.24)	0.74 (0.03)
Systolic BP	0.50 (<0.01)	0.34 (0.17)	0.61 (<0.01)	0.51 (0.19)
Diastolic BP	0.33 (0.02)	0.24 (0.36)	0.45 (0.02)	0.10 (0.82)

PCOS, Polycystic ovary syndrome; *IOH*, idiopathic ovarian hyperandrogenism; *IAH*, idiopathic adrenal hyperandrogenism; *SHBG*, sex hormone binding globulin; *BP*, blood pressure. All *p* values are in parentheses.

ian stroma of women with polycystic ovary syndrome and hyperthecosis have been found.[10] A feasible alternative mechanism for the altered lipid findings is that insulin working through insulin-like growth factor I receptor in the ovary may stimulate androgen production and secondarily affect lipid metabolism as opposed to insulin's well-known direct effects on liver metabolism. Low levels of HDL_2-cholesterol concentrations were found in all the subgroups. Concentrations of HDL_2 cholesterol fluctuate depending on modulation of hepatic lipase, which is known to be sex steroid responsive,[11] and the action of lipoprotein lipase, which is known to be insulin responsive.[12]

Excess body weight and android body fat distribution independent of the influence of obesity affect insulin resistance in nonhirsute women.[13] Concentrations of sex hormone binding globulin reflect androgen/estrogen[14] and insulin[15] influence. In women with polycystic ovary syndrome, fasting and 2 hour insulin concentrations were associated with waist/hip ratio and percent ideal body weight. In women with idiopathic ovarian hyperandrogenism, the androgen, insulin, and blood pressure variables were associated with percent ideal body weight and waist/hip ratios (Table IV and V). This is of interest because higher waist/hip ratios may reflect androgen influence.[16, 17] Waist/hip ratio is a useful predictor of diseases of the circulatory system.[18] It is likely that these differing associations in women with polycystic ovary syndrome versus idiopathic ovarian hyperandrogenism reflect differing pathophysiologic mechanisms or alternatively similar pathophysiologic influence viewed at different times during development.

Recent epidemiologic data suggest that hyperinsulinism is the initial abnormality that accounts for the association of ischemic heart disease with glucose intolerance, obesity, and hypertension. Hyperinsulinemia is associated with a higher risk of coronary vascular disease than obesity, glucose intolerance, or even diabetes. Healthy persons with hyperinsulinemia and normal glucose tolerance may have an increase in risk factors for cardiovascular disease as compared with a well-matched group of healthy subjects with normal insulin levels.[19] It is possible that women with idiopathic ovarian hyperandrogenism display the insulin resistance X syndrome referred to by Reaven.[20] With aging and long-standing insulin resistance there is elevated insulin, elevated blood pressure, elevated androgen, elevated triglyceride, and lowered HDL cholesterol levels. It is possible that DHEAS confers protection

against death, cardiovascular disease in particular,[21] through disturbing this relationship. Clearly, the relationships between insulin resistance, blood pressure, and lipoprotein lipid metabolism in various subgroups of hyperandrogenic women need further investigation.

Whereas the exact mechanism(s) as to why these alterations occur remains to be elucidated, our studies suggest that women with hyperandrogenism are at increased risk of coronary vascular disease. The insulin resistance, the increased waist/hip ratios, and the altered lipoprotein lipid metabolism that some of these women display, coupled with sedentary life styles,[1] hypertension,[22] and diets low in fiber and high in saturated fats[1] may place some of them at risk of coronary vascular disease. Hirsutism, a common manifestation of androgen excess may signal that risk.[7]

REFERENCES

1. Wild RA, Painter PC, Coulson PB, Carruth LB, Ranney GB. Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1985;61:946-51.
2. Wild RA, Bartholomew MS. The influence of body weight on lipoprotein lipids in patients with polycystic ovary syndrome. AM J OBSTET GYNECOL 1988;159:423-7.
3. Wild RA, Applebaum-Bowden D, Demers LM, et al. Lipoprotein lipids in women with androgen excess: independent associations with increased insulin and androgen. Clin Chem 1990;36(2):283-9.
4. Chetkowski RJ, DeFagio J, Shamonki IM, Judd HL, Chang RJ. The incidence of late-onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency among hirsute women. J Clin Endocrinol Metab 1984;58:595-8.
5. Kirschner MA, Samojlik E, Drejka M, Szmal E, Schneider G, Ertel N. Androgen-estrogen metabolism in women with upper body versus lower body obesity. J Clin Endocrinol Metab 1990;70:473-9.
6. New MI, White PC, Pang S, Dupont B, Speiser PW. The adrenal hyperplasias. In: Scriver CR, Beaudet AL, Sly WL, Valle D, eds. The metabolic basis of inherited disease 6th ed. New York: McGraw-Hill, 1989.
7. Santen RJ, Bardin W. Episodic LH secretion analysis in man: clinical interpretation and physiological mechanisms. JCI 1973;52:2617-28.
8. Dunaif A, Graf M, Mandeli J, Laumas V, Dohrjansky A. Characterization of groups of hyperandrogenic women with acauthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. J Clin Endocrinol Metab 1987; 65:499-507.
9. Poretsky L, Kalin M. The gonadotropic function of insulin. Endocr Rev 1987;8(2):132-41.
10. Nagamani M, Stuart CA. Specific binding sites for insulin-like growth factor I (IGF-I) in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis. [Abstract 409]. In: Proceedings of the thirty-seventh annual meeting of the Society for Gynecologic Investigation. St. Louis: Society for Gynecologic Investigation, 1990.
11. Sorva R, Kiwsit, Taskinen MR, Percheentupa J, Nikkila EA. Testerone substitution increases the activity of lipoprotein lipase and hepatic lipase in hypogonadal males. Atherosclerosis 1988;69:191-7.
12. Yki-Jarvinen H, Taskinen M, Koivisto VA, Nikkila EA. Response of adipose tissue lipoprotein lipase activity and serum lipoproteins to acute hyperinsulinemia in man. Diabetol 1984;26:364-9.
13. Kissebah AH, Freedman DS, Peiris AN. Health risks of obesity. Med Clin North Am 1989;73:111-38.
14. Anderson DC. Sex-hormone-binding globulin. Clin Endocrinol 1974;3:69-74.
15. Plymate S, Matej LA, Jones RE, Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (HepG2) cell line by insulin and prolactin. J Clin Endocrinol Metab 1988;67:460-4.
16. Evans DJ, Hoffman RG, Kalkhoff RG, et al. Relationship of androgenic activity to body-fat topography, fat-cell morphology, metabolic alterations in premenopausal women. J Clin Endocrinol Metab 1983;57:304-9.
17. Wild RA, Grubb B, Hartz A, Von-North J, Bachman W, Bartholomew MS. Clinical signs of androgen excess as risk factors for coronary artery disease. Fertil Steril 1990; 54(2):001-5.
18. Lapidus L, Bengtsson C, Larsson B, et al. Distribution of adipose tissue and risk of cardiovascular disease and death; a 12-year follow-up of participants in the population study of women in Gothenberg, Sweden. Br Med J 1984;289:1257-66.
19. Zavaroni I, Bonora E, Pagliara M, et al. Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N Engl J Med 1989;320:702-6.
20. Reaven GM. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
21. Barrett-Connor E, Khaw KT, Yen SC. A prospective study of dehydroepiandrosterone sulfate mortality and cardiovascular disease. N Engl J Med 1986;315:1591-24.
22. Mattson LC, Gullberg G, Hamberger L, Samsiol G, Silfuerstolpe G. Lipid metabolism in women with polycystic ovary syndrome: possible alterations for an increased risk of coronary artery disease. Fertil Steril 1984;42:479-84.

Lectins and heparin-binding features of human spermatozoa as analyzed by flow cytometry

Jaime M. Vasquez, MD,[a, c] Susan F. Magargee, MS,[b] Elaine Kunze, BA,[b] and Roy H. Hammerstedt, PhD[b]

Madison, Wisconsin, and University Park and Philadelphia, Pennsylvania

Sperm cell surface characteristics may serve as a useful diagnostic feature for the evaluation of seminal samples, but to date no useful analytical method(s) is available for their routine quantitative analysis. Fluorescein isothiocyanate–conjugated lectins, proteins with unique and specific saccharide-binding characteristics, and heparin are potentially useful markers for such an analytical procedure. A protocol is outlined whereby frozen semen is thawed and treated with either fluorescein isothiocyanate–conjugated soybean agglutinin SBA *(Glycine max)*, peanut agglutinin PNA *(Arachis hypogaea)*, and garden pea agglutinin PSA *(Pisum sativum)* lectins or fluorescein isothiocyanate–heparin to assess differences in spermatozoa-bound fluorochrome by flow cytometry. Use of propidium iodide to identify nuclei containing sperm cells within the ejaculate is essential to the method because of the large amount of debris associated with the samples. The precision of the assay is high, with the coefficient of variation for the individual probes being <20%. Analysis of a patient pool of 22 revealed that a range in binding properties is observed and a mode of grouping these samples into classes for later analysis is outlined. Semen parameters and fluorescein isothiocyanate–ligand binding values were different in a population of unproven fertility as compared with a comparable population of known fertility. A positive correlation ($p < 0.001$) for garden pea agglutinin PSA binding and concentration of motile cells, percentage of motility, and number of motile cells was found. These data suggest the possible utility of flow cytometry for the assessment of human seminal samples. (Am J Obstet Gynecol 1990;163:2006-12.)

Key words: Lectins, heparin, human spermatozoa, flow cytometry

An elusive goal of andrology research has been the development of laboratory-based assays that possess a predictive capacity for evaluation of the fertility of semen samples; to date no such method(s) has been developed. Reasons are not apparent but may be related to a necessity for a multivariate analysis, with each aspect directed to the evaluation of a unique and obligatory aspect of the functional sperm cell. Reasonable candidates include anlaysis of motility, acrosomal status, and sperm surface features related to sperm binding to the ovum.[1]

Progress toward such a pattern of analysis is being made in that reasonable candidates for sperm functionality have been identified and some quantitative assays have been developed.[1-4] Techniques and instruments for computer-assisted analysis of spermatozoal motility are available, although appropriate incubation and sample preparation techniques for retention (or induction) of motility characteristics appropriate for predictive analysis have yet to be identified. Even less progress has been made in the quantitation of sperm surface characteristics related to successful sperm-ovum binding and acrosomal activation.

Fluorescein isothiocyanate–lectins, high molecular weight glycoproteins with an affinity for saccharide molecules, have been used in conjunction with flow cytometry as probes for terminal saccharides on the surface membranes of ram sperm.[5] Heparin, another glycoprotein whose binding to bull sperm has been tested for correlation to fertility, also may serve as a potential ligand for classification of human spermatozoal surface features.[1, 6, 7]

We describe herein an attempt to classify human sperm by quantitation of binding properties of individual cells by flow cytometry. Because a quantitative evaluation of fertilization potential of human sperm samples is difficult, attention in preliminary studies is best directed to screening samples for those descriptors that reflect extremes in the population. The underlying

From the Department of Obstetrics and Gynecology, University of Wisconsin Medical School–Madison,[a] the Department of Molecular and Cell Biology, The Pennsylvania State University,[b] and the Department of Obstetrics and Gynecology, Thomas Jefferson University and Medical School.[c]

Supported by a Biomedical Research Support Grant to the University of Wisconsin Medical School from the National Institutes of Health, Division of Research Facilities and Resources, Bethesda, Maryland, (J. M. V.), a University of Wisconsin Graduate School Grant (J. M. V.), and National Institutes of Health, National Institute of Child Health and Development Grant No. HD-13099 (R. H. H.).
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: J. M. Vasquez, MD, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Thomas Jefferson University and Medical School, 1025 Walnut St., Room 310, Philadelphia, PA 19107.
6/6/24721

assumption of this approach is that a validated quantitative analysis of semen samples from an unscreened (i.e., unknown fertility) population should allow grouping of the population into two or three classes related to the detection of outliers from the population mean. Subsequent studies then can focus on a more refined set of descriptors in a detailed comparison of spermatozoal features and measurements of fertility.

Material and methods

After obtaining the appropriate Human Subject's Committee clearance, semen was collected from 22 males who requested infertility evaluation and one control. The semen was allowed to liquify and was subjected to computer analysis. The control subject was a donor for the artificial insemination program and his fertility had been documented. The control subject provided eight semen samples for flow cytometric analyses at regular intervals throughout the study period. These semen samples were collected and analyzed in parallel with the infertility population. Aliquots were then removed and frozen at −196° C. Frozen sperm samples were rapidly thawed in the presence of 70% ethanol (added 1:1, vol:vol), centrifuged at 300 *g* for 10 minutes, and resuspended in Hanks' balanced salt solution, pH 7.2, buffered with 5 mmol/L HEPES buffer. Cells were diluted to a concentration of 4×10^7 cells/ml.

Computer-assisted semen analysis. Sperm concentrations were evaluated with a Cellsoft automated semen analyzer (CRYO Resources, Ltd., New York, N.Y.). Other sperm traits analyzed included total cell number, concentration of motile cells, percentage of motility, velocity mean, and linearity mean. Standard equipment (Olympus BH2 microscope, 10× objective, 6.7× photographic eyepiece, MTV-3 video camera adaptor, 10-unit Makler chamber) for operation of an automated semen analyzer was used. The general parameters for the semen analysis were as follows: frames, 20; Hz, 30; minimum sampling for motility, 1; minimum sampling for velocity, 3; maximum for velocity, 200; threshold velocity, 8; threshold gray, 115; Pixel scale, 0.688; dilution factor, 1; cell size range, 5 to 25. After computer analysis, aliquots were removed and frozen in 7.5% glycerol at −196° C or without glycerol.

Lectins and heparin. Fluorescein isothiocyanate–conjugated lectins were obtained from E-Y Laboratories (San Mateo, Calif.). The lectins used in this study were as follows: soybean agglutinin SBA *(Glycine max)* (binding specificity for N-acetyl galactosamine residues, and α-D-galactose), peanut agglutinin PNA *(Arachis hypogaea)* (binding specificity for β-D-galactose residues), and garden pea agglutinin PSA *(Pisum sativum)* (binding specificity for α-D-mannose). Fluorescein isothiocyanate–heparin was purchased from Polysciences (Warranton, Pa.). Extensive preliminary experiments (data not presented) established the following protocol. In a final volume of 0.5 ml, 0.05 ml cells (2×10^6) were added to fluorescein isothiocyanate–conjugated lectin (1 to 210 μg), 10 μg propidium iodide and brought to a final volume of 0.5 ml with Hanks' balanced salt solution. Cells were incubated at room temperature for 30 minutes. Preliminary tests indicated that lectin binding was maximum by this time and no further increase in cellular fluorescence was noted when longer incubation times were used.

Automated quantification by flow cytometry. Fluorescence measurements were performed on a flow cytometer EPICS V, (Coulter Electronics, Hialeah, Fla.). The filters were arranged as follows: 515 nm laser blocking filter, 560 dichroic with an additional 515 nm long pass filter in front of the green photomultiplier tube (lectin-fluorescein isothiocyanate measurement) and a 590 long pass in front of the red photomultiplier tube [PI-deoxyribonucleic acid (DNA) measurement]. Photomultiplier high-voltage settings were adjusted so that 10 μm fluorescent microspheres (lot number 4009, Coulter Electronics) fell in channel 172 for the log integrated green fluorescence parameter. The amplifier gains were adjusted to place the maximum signal from these standards in the same channel (172), thus minimizing variability between experiments. The instrument-default set-threshold-gating value for the amplifiers was used during data acquisition. All measurements were gated on log integrated red fluorescence which is a measure of DNA, to eliminate debris and clumps from the lectin distribution. Under these operating conditions, no signal was detected when unstained cells were passed through the instrument. Thus all data displayed represent sperm with varying amounts of bound lectin or heparin. A total of 1×10^4 sperm cells with this gate were counted.

Results

The necessity of counterstaining and gating for propidium iodide-positive cells (DNA-containing) cannot be overemphasized. Typically, only 25% to 40% of each sample were DNA-containing particles. The remainder was debris.

To eliminate potential health hazards associated with semen that contains the frozen sperm human immunodeficiency virus (HIV), suspension was thawed in the presence of equal volume of 70% ethanol. Such treatment could displace glycoproteins from the sperm surface, but the practice was viewed as reasonable because the analysis involves formation of microdroplets during analysis by flow cytometry. In an experiment to compare fresh versus ethanol-treated bovine sperm (data not shown), neither the population distribution nor the total amount of fluorescein isothiocyanate–heparin bound per cell was altered by the ethanol treatment.

Table I. Differences in seminal properties and fluorescein isothiocyanate–conjugated ligand voltages between subjects with unproven fertility and control ejaculates (means ± SEM)

	Unproved fertility	*Control*	*p Value*
Concentration (million/ml)	60.8 ± 8.3	110.3 ± 13.3	0.0001
Concentration of motile cells (million/ml)	38.2 ± 8.3	59.8 ± 16.3	0.018
Motility (%)	50.3 ± 5.2	58.8 ± 6.3	0.12
Total no. cells per ejaculate (millions)	174.6 ± 28.5	231.8 ± 31.3	0.059
Velocity (μm/sec)	39.3 ± 1.9	43.7 ± 2.3	0.039
Linearity	5.2 ± 0.1	5.8 ± 0.2	0.0001
Abnormal morphology (%)	16.4 ± 2.3	19.6 ± 2.6	0.185
White blood cells* (/hpf)	6.4 ± 1.7	6 ± 1.5	0.806
pH	7.4 ± 0.07	7.3 ± 0.05	0.359
Volume	3.03 ± 0.38	3.14 ± 0.52	0.782
Heparin	0.11 ± 0.003	0.16 ± 0.002	0.0001
SBA	0.102 ± 0.005	0.179 ± 0.008	0.0001
PNA	0.113 ± 0.006	0.202 ± 0.007	0.0001
PSA	0.151 ± 0.01	0.335 ± 0.021	0.0001

SBA, Soybean agglutinin SBA; *PNA*, peanut agglutinin PNA; *PSA*, garden pea agglutinin PSA.
*Per high-power field.

A one-sample Student's *t*-test[17] was used to compare the differences in seminal properties and fluorescein isothiocyanate–ligand voltages between the patients with unproved fertility and the ejaculates obtained from the control (Table I). This revealed a significant difference in several semen parameters ($p < 0.05$) and all fluorescein isothiocyanate–ligands used ($p < 0.001$).

Linear regression models were used to compare fluorescein isothiocyanate–ligands bound to sperm and semen characteristics.[17] Data were analyzed with a minimum confidence level of 95%. Analysis of binding of fluorescein isothiocyanate–ligands relative to seminal properties determined by an automated semen analyzer revealed a positive correlation ($p < 0.0001$) for garden pea agglutinin PSA binding and percentage of motility, concentration of motile cells, and number of motile cells. A negative correlation ($p < 0.05$) for garden pea agglutinin PSA and percentage of abnormal cells was also found. The remaining seminal traits of ejaculate volume, sperm velocity, linearity, ejaculate pH, and white blood cell count were not correlated with the binding of any of the fluorescein isothiocyanate–ligands.

Preliminary experiments determined the concentration of lectin or heparin necessary to accurately estimate the surface determinants and thereby allow quantitative comparisons of semen samples. Nonspecific binding of lectins was evaluated (data not shown) by adding sugars that competitively displace the fluorescein isothiocyanate–lectin from the surface. The fluorescence associated with the sperm cells was reduced about tenfold by such treatment. Attempts to accomplish a similar test for heparin were unsuccessful because no true competitive ligand is available to displace any previously bound fluorochrome. The results (Fig. 1) of a titration of fluorescein isothiocyanate–lectin or heparin versus fluorescence intensity as assessed in the flow cytometer established that the following amounts of ligand were sufficient to allow estimation of the binding capacity of the surface of the cells: soybean agglutinin SBA and peanut agglutinin PNA, 70 μg; garden pea agglutinin PSA, 60 μg; and heparin, 100 μg. Tests of other lectins, castor bean agglutinin RCA *(Ricinus communis)* and concanavalin A agglutinin Con A *(Canavalia ensiformis)* established that accurate analyses could not be accomplished at lectin contents of <70 μg; therefore these ligands were not studied further. A typical distribution profile for one subject (assayed with the stated ligand concentrations) for the three lectins and heparin is provided in Fig. 2. An estimate of fluorescence intensity per sample for the 1×10^4 sperm analyzed is indicated by an asterisk. For all subsequent comparisons, this expression of the primary data for peak intensity (expressed as channel number and on a logarithmic scale) was converted to phototube voltage on a linear scale.

Once the appropriate level of lectin or heparin was established, a single semen sample from each of 22 subjects was analyzed. In addition, replicate analyses of eight semen samples obtained from a single subject with proved fertility over a 3-month period were made for comparison with the infertile population and to determine the variability in the assay (Fig. 3); the coefficient of variation for this subject was 4.5% for heparin, 13.9% for soybean agglutinin SBA, 10.1% for peanut agglutinin PNA, and 17.4% for garden pea agglutinin PSA.

To detect outliers in this set of semen samples, the mean fluorescence for each lectin was calculated for the

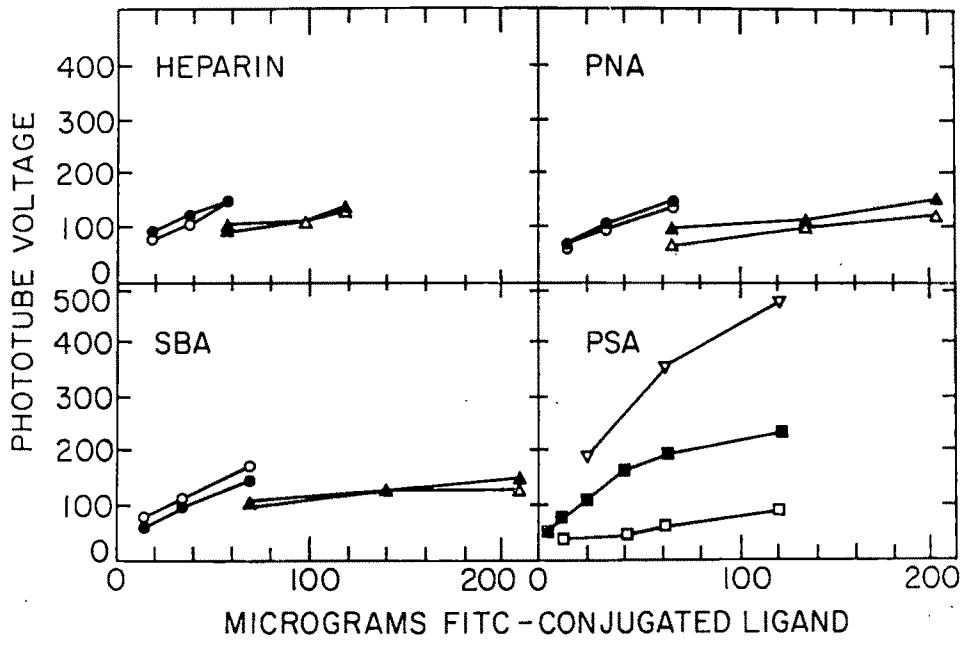


Fig. 1. Titration of sperm surfaces with increasing ligand concentration. Sperm were treated with increasing concentrations of fluorescein isothiocyanate–ligand and fluorescence intensity of nucleated particles in suspension was assessed as outlined in text. These data established that reproducible evaluation of extent of ligand binding to sperm could be accomplished for the four compounds presented. Those curves with identical symbols represent analyses of same sperm suspension with different ligands.

22 singly analyzed samples. The equation $[(X_n - X_{mean})/SD]$ was used to display the difference between the sample value (X_n) and the population mean (X_{mean}) in terms of the SD for that lectin (Fig. 4). For comparison, the control sample with its SD for eight replicate analyses is presented. The 22 samples were grouped into classes (Table II) according to their difference from the sample mean. The scheme used for subsequent comparisons was: group a, within 1 SD of the mean; group b, >1 SD but <2 SD from the mean; and group c, >2 SD from the mean.

An example of a subject with uniquely high binding capacity was subject 22. This subject had normal semen parameters according to our laboratory (concentration, 64.5 million/ml; percentage of motility, 57%; concentration of motile cells, 37.3 million/ml; abnormal forms, 22%).

Comment

The initiation of a study of potentially important descriptors of the sperm surface is critically dependent on the choice of ligand for the study. Our choices were made on the basis of previous results for human and ram sperm. Two ligands have been shown to be related to features known to be involved in successful sperm-ovum binding. Heparin binding to human sperm has been shown to be positively correlated with the capacity of such sperm preparations to bind and penetrate denuded hamster ova[1] whereas garden pea agglutinin PSA binding to human sperm has been shown to be directly related to extent of the acrosome reaction.[8, 9] Thus differences among subjects in these two parameters could be related to fertilization capacity. The other two ligands studied, soybean agglutinin SBA and peanut agglutinin PNA, were chosen because previous studies of ram sperm revealed changes in their binding capacity associated with physiologic states; however, no direct relationship to fertilization capacity has been inferred from previous data.

The large amount of debris (e.g., nonnucleated particles) in human semen presents a special challenge to analysis by flow cytometry. Use of propidium iodide to select (gate on) particles for analysis is critical because some of the debris binds lectins and heparin. As an example, when heparin binding to all particles was analyzed at least three populations of fluorescent particles were detected rather than the single distribution shown in Fig. 2.

In our study, samples from the patients and the control subject were collected throughout the study period but were assayed simultaneously to avoid interassay variation and statistical bias. This required storing frozen samples until the assays were performed. In addition, because of obvious reasons, the use of frozen samples was the only way in which two laboratories from different parts of the country could collaborate in this project.

It is true that the use of frozen-thawed sperm may

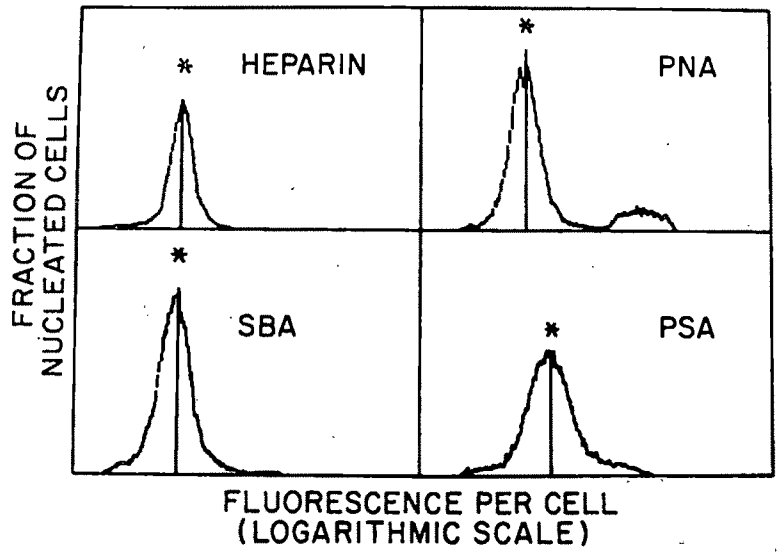


Fig. 2. Binding properties of ligands to sperm within a seminal sample. When sperm were assayed under optimum concentrations of fluorescein isothiocyanate–ligand (see text), homogeneous distribution of amount of fluorescence per cell (logarithmic scale; X axis) versus percent of population with nucleated particles (sperm; linear scale; Y axis) was detected for each ligand. Position of major peak in each distribution on the basis of analysis of 1×10^4 sperm, is marked with *asterisk;* this fluorescence value was used as descriptor for entire population in subsequent calculations.

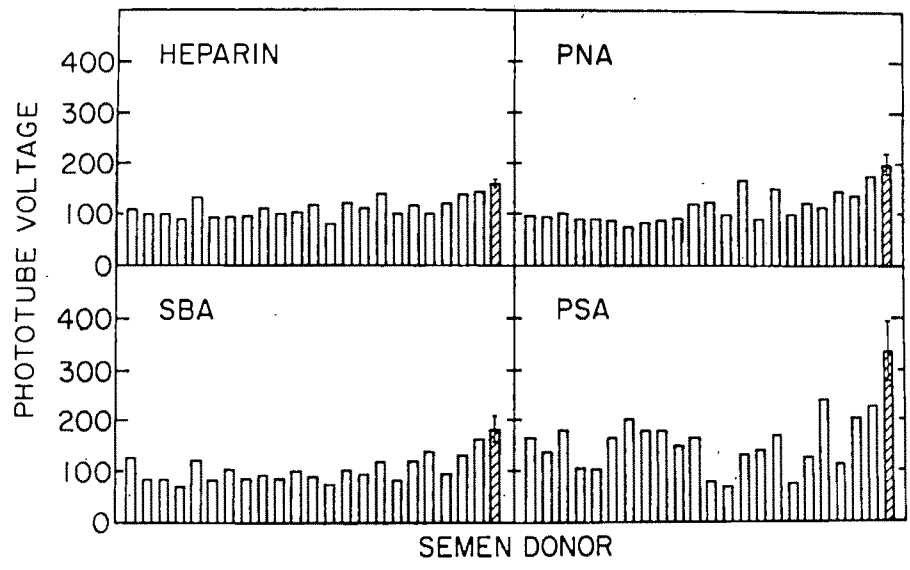


Fig. 3. Analysis of ligand binding properties of individual ejaculates by flow cytometry. Single ejaculates from 22 men were analyzed as outlined in text. Fluorescence intensity of major peak (*asterisk,* Fig. 2) was transformed from channel number (logarithmic scale) to linear scale (voltage) for direct comparison in this plot. *Last bar to right (cross-hatch)* represents mean value (± 1 SD) for analysis of eight ejaculates from one control subject.

lead to the release or exposure of internal components from their membranes. However, the Center for Disease Control and the American Fertility Society have recommended the exclusive use of frozen semen as an important safeguard for patients.[10, 11] Thus it is important to develop techniques to quantitatively assess potential for fertility of cryopreserved human semen samples, although the conclusions drawn from these descriptors may not necessarily apply to fresh spermatozoa.

Although we noted that in fresh versus ethanol-treated bovine spermatozoa there was no difference in the amount of ligand bound per cell or in the population distribution, this experiment could not be carried

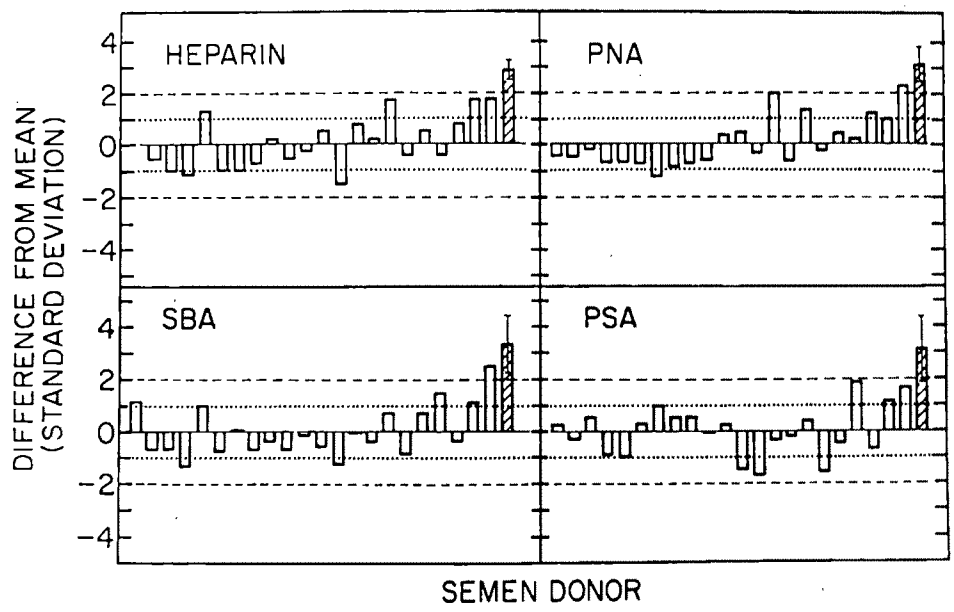


Fig. 4. Normalization of ligand binding properties of individual ejaculates. Data of Fig. 3 were normalized relative to mean binding properties of the 22 samples analyzed. Expression of difference in total binding for each sample from that of mean (in terms of SD for binding of that ligand) allows direct comparison within subjects across ligands to be tested. *Light line* represents ± 1 SD and *heavy line* represents ± 2 SD. Population mean ± SEM (expressed in volts output from detector per cell analyzed) for each lectin were as follows: heparin, 0.110 ± 0.018; soybean agglutinin SBA, 0.102 ± 0.023; peanut agglutinin PNA, 0.113 ± 0.047; garden pea agglutinin PSA, 0.151 ± 0.047. Last bar *(cross-hatch)* represents mean value (± 1 SD) for analysis of eight ejaculates from one subject and was not included in population mean.

out with human spermatozoa. In the past, some investigators performed fluorescence-activated cell sorting of human spermatozoa without ethanol treatment.[13] However, this practice is now considered unsafe because HIV has been isolated from the semen of patients with HIV after the samples were cryopreserved in liquid nitrogen.[14] An experiment comparing fresh versus ethanol-treated human spermatozoa would defy current recommendations from the Center for Disease Control to prevent the formation of HIV-containing microdroplets.[15, 16]

The lectin-binding properties of human sperm are remarkably constant and homogeneous. Under the conditions used, a single major population was detected. For the sample assayed to establish repeatability, the fluorescence intensities of the individual ejaculates were highly reproducible with a coefficient of variation of <20% approaching that expected from standard gamete biology laboratory assays. Such reproducibility should allow detection of those sperm preparations that possess unique binding features.

Sperm preparations with uniquely high or low binding capacities for the ligands tested were detected (Fig. 4 and Table II). Over half the samples analyzed had binding properties within 1 SD of the mean (group a of Table II). Assays with both the ligands with unknown relation to fertilization capacity (soybean agglutinin SBA and peanut agglutinin PNA) and the pair (heparin

Table II. Relationship of ligand binding to surfaces of human sperm

		Ligand tested		
Patient	*Heparin*	*SBA*	*PNA*	*PSA*
1	a	+b	a	a
2	a	a	a	a
3	−b	a	a	a
4	−b	−b	a	a
5	+b	+b	a	a
6	a	a	a	a
7	a	a	−b	+b
8	a	a	a	a
9	a	a	a	a
10	a	a	a	a
11	a	a	a	a
12	a	a	a	−b
13	−b	−b	a	−b
14	a	a	+b	a
15	a	a	a	a
16	+b	a	+b	a
17	a	a	a	−b
18	a	a	a	a
19	a	+b	a	+b
20	a	a	+b	a
21	+b	+b	a	+b
22	+b	+c	+c	+b

SBA, Soybean agglutinin SBA; *PNA*, peanut agglutinin PNA; *PSA*, garden pea agglutinin PSA.

Binding properties of sperm were assessed from the data presented in Fig. 4 and classified according to the scheme presented in the text. A plus or minus sign indicates a value above or below the population mean, respectively.

and garden pea agglutinin PSA) previously related to essential features of sperm-ova interactions, revealed individual samples with uniquely high or low binding capacities. For example, subject 22 had high binding capacity especially for soybean agglutinin SBA and peanut agglutinin PNA, and this subject had normal semen parameters. Also note that in the control subject the binding values for all the probes, including heparin, were >2 SDs from the infertile population mean. At this time the significance of such variation is unknown, but the ability to precisely detect such variants is essential to an eventual test of any relation to fertilization capacity of the sperm preparation.

A statistical test of the ligand-binding variables with results of analysis by an automated semen analyzer of seminal properties before freezing revealed a few relationships: a positive correlation ($p < 0.0001$) for garden pea agglutinin PSA binding and concentration of motile cells, percentage of motility, and number of motile cells. The significance of these is not apparent because no relationship of motility to a surface property has been established. However, a positive correlation between these seminal properties and fertilization of human oocytes in vitro has been shown by other investigators.[12]

Fluorescein isothiocyanate–lectin and fluorescein isothiocyanate–heparin binding has not been related to fertility in human beings. However, the correlations between these fluorescein isothiocyanate–ligands and several sperm physical and morphologic traits suggest the possible use of flow cytometry to assess human semen. In addition, the differences in fluorescein isothiocyanate–ligand measurements between patients and control ejaculates indicate that the binding of these probes may be a predictor of fertility in human beings. Further research is warranted to establish the usefulness of this assay, particularly in a clinical context.

REFERENCES

1. Vasquez JM, Winer M, Ax RL, Boone WR. Correlation of human spermatozoa heparin binding with the zona-free hamster egg in vitro penetration assay. AM J OBSTET GYNECOL 1989;160:20.
2. Vasquez JM, Lavin CA, Boone WR, Schatten G. Monitoring of capacitation by the chlortetracycline fluorescence assay and its relation to penetration of zona-free hamster eggs [Abstract]. In: Proceedings of the thirty-sixth annual meeting of the Society for Gynecologic Investigation, March 15-18, 1989; San Diego, California: Society for Gynecologic Investigation, 1989.
3. Vasquez JM, Kunze E, Magargee SF, Boone WR, Hammerstedt RH. Lectin-binding features of human spermatozoa as analyzed by flow cytometry [Abstract]. In: Proceedings of the thirty-sixth annual meeting of the Society for Gynecologic Investigation, March 15-18, 1989. San Diego, California: Society for Gynecologic Investigation, 1989.
4. Vasquez JM, Lavin CA, Parrish JJ, Winkel CA. Correlation between capacitation determined by chlortetracycline (CTC) fluorescence and physical traits of human sperm [Abstract]. In: Proceedings of the thirty-seventh annual meeting of the Society for Gynecologic Investigation, March 21-24, 1990. St. Louis, Missouri: Society for Gynecologic Investigation, 1990.
5. Magargee SF, Kunze E, Hammerstedt RH. Changes in lectin-binding features of ram sperm surfaces associated with epididymal maturation and ejaculation. Biol Reprod 1988;38:667.
6. Vasquez JM, Miller DJ, Shapiro SS, Ax RL. Correlations between ^{3}H-heparin binding and physical traits of human sperm [Abstract]. In: Proceedings of the thirty-fourth annual meeting of the Society for Gynecologic Investigation, March 18-21, 1987. Atlanta: Society for Gynecologic Investigation, 1987:47.
7. Miller DJ, Agnew G, Boone WR, Ax RL, Vasquez JM. Relationship of heparin binding with computer-analyzed physical traits of human sperm. Fertil Steril 1988;49:886.
8. Cross NL, Morales P, Overstreet JW, Hanson FW. Two simple methods for detecting acrosome reacted human sperm. Gamete Res 1986;15:213.
9. Cross NL, Overstreet JW. Glycoconjugate of the human sperm cell surface: distribution and alterations that accompany capacitation in vitro. Gamete Res 1987;16:23.
10. Center for Disease Control. Semen banking, organ and tissue transplantation, and HIV antibody testing. MMWR 1988;37:57.
11. American Fertility Society. Revised new guidelines for the use of semen-donor insemination. Fertil Steril 1988;49:211.
12. Holt WV, Moore HDM, Hillier SG. Computer-assisted measurement of sperm swimming speed in human semen: correlation of results with in vitro fertilization assays. Fertil Steril 1985;44:112.
13. Tyler JPP, Dobler KJ, Driscoll GL, Stewart GJ. The impacts of AIDS on artificial insemination by donor. Clin Reprod Res 1986;4:305.
14. Hummel WP, Talbert LM. Current management of a donor insemination program. Fertil Steril 1989;51:919.
15. Resnick L, Veren K, Salahuddin SZ, Tondreau S, Markham PD. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. JAMA 1986; 255:1887.
16. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987;36:5S.
17. Daniel WN (ed). In: Biostatistics: a foundation for analysis in the health sciences. New York: J Wiley and Sons, 1978.

Preembryo biopsy and analysis of blastomeres by in situ hybridization

Jamie A. Grifo, MD, PhD,[a,*] Ann Boyle, BS, MS,[b] Evan Fischer, BA,[a] Gad Lavy, MD,[a] Alan H. DeCherney MD,[a] David C. Ward, PhD,[b] and Mrinal K. Sanyal, PhD[a]

New Haven, Connecticut

We developed a method for the biopsy of preimplantation mouse embryos (preembryos) at the four- to eight-cell stage, which uses partial zona pellucida dissection. The preembryos were collected in calcium- and magnesium-free phosphate-buffered saline solution with 0.01% ethylenediaminetetraacetic acid, 0.1 mol/L sucrose, and 4 mg/ml of bovine serum albumin to facilitate removal of blastomeres. This allows entry of a fine micropipette into the perivitelline cavity with subsequent removal of a single blastomere by gentle suction. The majority of embryos (75%) from which biopsy specimens were obtained in this fashion developed to the blastocyst stage. The blastomeres obtained were mainly intact and they were fixed to glass slides. After permeabilization, in situ hybridization was performed with chromosome X– and chromosome 3–specific probes. Human unfertilized eggs and blastomeres from human polyspermic embryos also have been analyzed by in situ hybridization with chromosome specific probes. The combination of nondestructive embryo biopsy and in situ hybridization is a possible approach for preimplantation genetic diagnosis. (Am J Obstet Gynecol 1990;163:2013-9.)

Key words: Preembryo biopsy, in situ hybridization, blastomere

Recent advances in molecular genetics have improved the sensitivity of methods used for genetic diagnosis. Two techniques in particular, the polymerase chain reaction and in situ hybridization permit the analysis of the genetic material of a single cell. The sensitivity of polymerase chain reaction is achieved by enzymatic amplification of target deoxyribonucleic acid (DNA) sequences by a factor of 10^5-fold or more.[1] In contrast, in situ hybridization allows the direct visualization of single genes in metaphase chromosomes or interphase nuclei.[2,3] The micromanipulation of preembryos could allow specific preimplantation diagnoses to be made. Currently, genetic diagnoses are limited to the postimplantation period and are performed by chorionic villus sampling or amniocentesis. If genetic diagnoses could be achieved in preimplantation embryos with no untoward effects then in vitro fertilization technology could be used for fertile couples at genetic risk. Because a broad range of diseases can be diagnosed by polymerase chain reaction or in situ hybridization, the technique of in vitro fertilization coupled with preembryo biopsy could provide the conduit from which these technologies can be applied to the human species.

Edwards[4] first suggested that sex-linked recessive disorders could be diagnosed by sampling trophoblast cells removed from blastocysts. Sex of bovine preembryos has been determined by chromosome analysis[5,6] or Y chromosome–specific DNA probes.[7] Monk et al.[8] have diagnosed a deficiency of the X-linked enzyme hypoxanthine phosphoribosyl transferase in mouse preembryos before transfer. However, analyses of this nature are limited to enzyme deficiency diseases that can be detected by microenzyme assay. Trophectoderm biopsy has been successfully carried out in marmoset monkey. These cells were cultured for analysis and the embryos were transferred with resultant live-born offspring.[9] Sex of the human preembryo has been successfully determined with chromosome-specific probes and in situ hybridization. However, this used a tritiated Y chromosome–specific probe that required a long time for diagnosis and the analysis used the whole embryo in a destructive fashion.[10] Whereas this method is successful, it would provide no clinical application. Recently, Handyside et al.[11] determined the sex of the human preembryo in a nondestructive fashion with embryo biopsy in conjunction with polymerase chain reaction. They were able to remove a single blastomere, amplify Y-specific DNA repeat sequences, and culture embryos from which biopsy specimens were obtained to blastocysts at the same rate as that of embryos from

From the Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology,[a] and the Department of Human Genetics,[b] Yale University School of Medicine.
Supported by National Institutes of Health Grant No. GM 41596 and the American College of Obstetricians and Gynecologists–Ortho Fellowship Award.
Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990.
Reprint Requests: Jamie A. Grifo, MD, PhD, The Center for Reproductive Medicine, The New York Hospital–Cornell University Medical Center, 525 E. 68th St., New York, NY 10021.
**American College of Obstetricians and Gynecologists—Ortho Fellowship Award recipient.*
6/6/24750

which biopsy specimens were not obtained. Holding and Monk[12] diagnosed β-thalassaemia by polymerase chain reaction in single blastomeres from mouse preimplantation embryos. The unique feature of this study is that they were able to amplify a region of a gene in a single diploid blastomere by a method that is theoretically applicable to any single copy genetic disorder in which a mutation or deletion is the cause. Wilton and Trounsen[13] recently described a method of mouse preembryo biopsy with successful cryopreservation. In addition, they developed a method for culture of blastomeres that after 6 days resulted in 20-cell nuclei per cultured blastomere.[14] Whereas in situ hybridization can be applied to a single cell, the ability to culture blastomeres would facilitate a more reliable diagnosis. In situ hybridization allows numeric chromosome analysis and is not limited by contamination. These are distinct advantages over polymerase chain reaction for analysis of single cells.

In this article we describe a method of embryo biopsy that uses partial zona dissection and we describe the first successful in situ hybridization with blastomeres obtained by embryo micromanipulation. In addition, we performed in situ hybridization with both unfertilized human eggs and with polyspermic human embryos.

Material and methods

Embryo collection. Female mice B6xC3/F1 were superovulated by intraperitoneal injections of 5 IU of pregnant mare serum gonadotropin (Gestyl, Diosynth) followed 48 hours later by 5 IU of human chorionic gonadotropin (Pregnyl, Organon) and mated with male B6xC3/F1 mice. Mated females were killed approximately 48 hours later and embryos were flushed from the oviducts with either Hoppe-Pitts media or phosphate-buffered saline solution with 4 mg/ml bovine serum albumin. Embryos were obtained at the two-cell to four-cell stage and 80% to 90% achieved the blastocyst stage when cultured in our system. Four- to eight-cell embryos were incubated for 30 minutes in calcium- and magnesium-free phosphate-buffered saline solution with 0.01% sodium ethylenediaminetetraacetic acid (EDTA) to reduce intercellular contacts. They were transferred to a small drop on a Lab-Tek microculture slide with the plastic chamber removed and covered with paraffin oil equilibrated with phosphate-buffered saline solution. Human polyploid embryos and oocytes that failed to fertilize were obtained from the in vitro fertilization program (Human Investigation Protocol 5443).

Micromanipulation. A single blastomere was removed from four- to eight-cell embryos with an inverted microscope and Narishige micromanipulators (Fig. 1). A holding pipette of approximately 80 μmol/L outside diameter with a 30 μmol/L inside diameter was fire polished with a Nikon microforge. A sharpened dissection pipette was used for zona dissection and a microbiopsy pipette was pulled to a 10 to 20 μmol/L outside diameter with a 5 to 15 μmol/L inside diameter. The preembryo was stabilized with gentle suction through the holding pipette and a slit was made through the zona pellucida with a sharpened dissection pipette similar to the method of Malter and Cohen.[15] The dissection pipette was removed and replaced by a biopsy pipette that was placed into the zona slit, and a blastomere was aspirated partially into the pipette under controlled suction. The blastomere was removed from the zona and the preembryo was placed into the Hoppe-Pitts media for continued culture after three passes into fresh media droplets. Embryo culture was done in microdrops under paraffin oil equilibrated with media.

Analysis of blastomeres. Blastomeres obtained in media droplets under oil were fixed directly onto the tissue culture slides under a Zeiss dissecting microscope in the following fashion. Two fixation protocols were used, one with 4% paraformaldehyde in 1/2X phosphate-buffered saline solution the other with 0.075 mol/L potassium chloride solution to swell the cell. Media was replaced with 4% paraformaldehyde in 1/2X phosphate-buffered saline solution or 0.075 mol/L potassium chloride and blastomeres incubated for 15 to 30 minutes. The oil was removed by suction and the solution surrounding the blastomere was removed without dislodging it. As the blastomeres started to air dry methanol/acetic acid (3:1) was dropped on to further fix them to the slide. This last step was repeated after air drying and finally 100% methanol was applied to remove excess oil and debris. Blastomeres were stored fixed at 4° C. Later experiments showed no need for paraformaldehyde fixation and this step was omitted. When this was done no permeabilization was required either. Human oocytes and polyploid embryos were processed in a manner similar to mouse preembryos; however, polyploid preembryos were not cultured after biopsy because all blastomeres were fixed to glass slides. The polyploid embryo shown in Fig. 2 had five blastomeres in it and only three were retained on the glass slide after the in situ hybridization procedure.

Permeabilization. Blastomeres were equilibrated in phosphate-buffered saline solution with 20% glycerol for 30 minutes and then 0.5% triton (vol/vol), 0.5% saponin (wt/vol) in phosphate-buffered saline solution for 10 minutes. The slides were immersed in liquid nitrogen for 5 seconds and allowed to thaw. Two additional freeze-thaw steps were performed. This permeabilized the cell to allow biotinylated DNA probes access to the nucleus during in situ hybridization. When it was omitted variable results were obtained with in situ hybridization in paraformaldehyde-fixed blas-

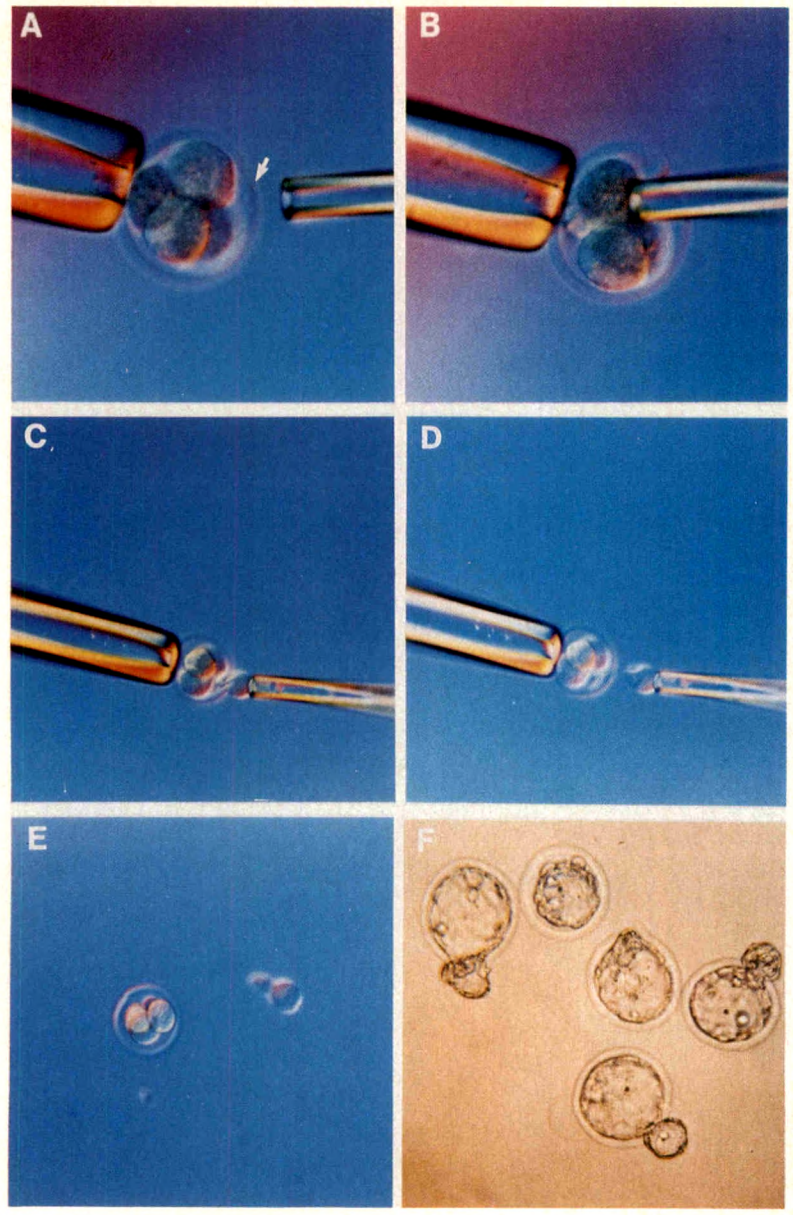


Fig. 1. Preembryo biopsy with zona dissection and removal of blastomere. **Panel A,** Four-cell mouse preembryo with slit in zona is immobilized on holding pipette. **Panel B,** Biopsy pipette is placed gently inside zona pellucida. **Panel C,** Gentle aspiration is used to remove blastomere from zona pellucida. **Panel D,** Blastomere is completely removed. **Panel E,** Embryo and blastomere are released into media. **Panel F,** Hatching blastocysts obtained from biopsied preembryos from which biopsy specimens were obtained.

tomeres. Later experiments showed that swelling the cells with 0.075 mol/L potassium chloride followed by fixation with acetic acid/methanol (1:3) also achieved adequate permeabilization.

DNA probes. The X repeat probe 60-36, a 4.2 kilobase (kb) insert was kindly provided by Dr. Christine Disteche.[16] It is a 4.2 kb sequence repeated approximately 50 times and maps just below and is not within the centromere. Probe MαG-6, a cosmid containing the genomic sequence for the sodium/potassium adenosine triphosphatase gene was provided by Dr. David Housman. It is a 40 kb insert that hybridizes the α-subunit single-copy gene.[17] The human Y repeat probe hybridizes to pHY2.1, a 2.1 kb sequence that is repeated approximately 2000 times on the long arm of the Y chromosome.[18] Probes were labeled with biotinylated-11-dUTP by nick translation.[2] The probes were nick translated intact with both the vector and insert and used in this form for in situ hybridization.

In situ hybridization. The X repeat probes and MαG-6 probes were used at a concentration of 6 μg/ml, which are standard amounts for in situ hybrid-

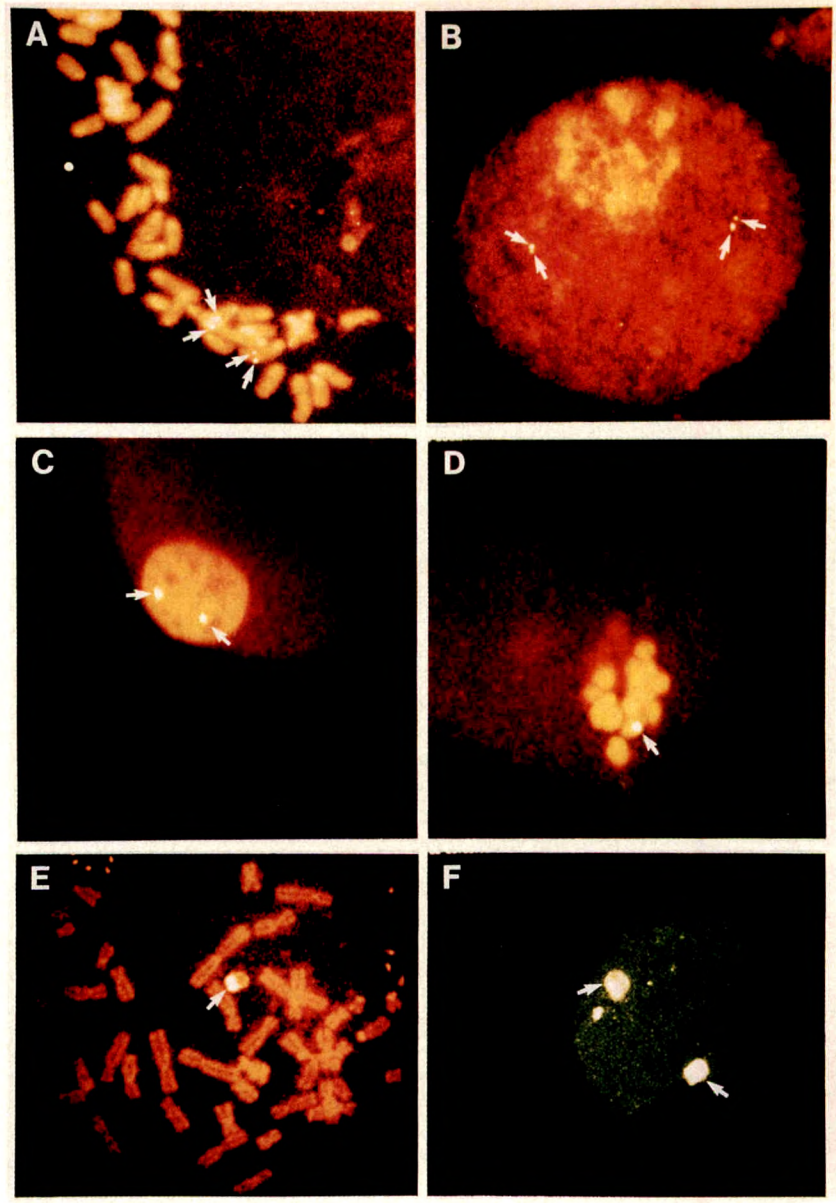


Fig. 2. Panel A, In situ hybridization to metaphase spread with cosmid clone that maps to chromosome 3 (sodium-potassium adenosine triphosphatase gene. **Panel B,** In situ hybridization to mouse blastomere with cosmid clone that maps to chromosome 3 (sodium-potassium adenosine triphosphatase gene). **Panel C,** In situ hybridiztion to mouse blastomere with X-specific repeat probe. Two signals are present consistent with XX karyotype. **Panel D,** In situ hybridization to mouse blastomere with X-specific repeat probe. This blastomere is in metaphase and has one signal. **Panel E,** In situ hybridization to human metaphase spread with Y-specific repeat probe; one signal is noted. **Panel F,** In situ hybridization with Y-specific repeat probe to blastomere from polyspermic human preembryo that has two major signals consistent with XYY karyotype. Two other cells from this preembryo also had two signals each.

ization performed in our laboratory. In addition to salmon sperm DNA, mouse total genomic DNA was added to suppress repetitive sequences in the probe DNA. The probe mixtures contained 20% dextran sulfate and 2X sterile saline citrate. Slides and probe were denatured separately. After denaturation for 2 minutes at 70° C in 20% formamide/2X sterile saline citrate, slides were dehydrated through a cold ethanol series and allowed to air dry. Probe mixtures were denatured at 75° C for 5 minutes followed by ice (for repeat probes) or a 37° C incubation for probes that required suppression.[2] Ten microliters of denatured probe was applied to the slide, coverslips were applied and sealed, and the slides were incubated overnight at 37° C. Slides were incubated for 5 minutes three times each in 50% formamide/2X sterile saline citrate at 42° C, then 5

minutes at 60° C three times in 0.1X sterile saline citrate. They were blocked for 30 minutes at 37° C in 3% bovine serum albumin, 4X sterile saline citrate, and 0.1% Tween 20 polysorbate preparation. Biotinylated probes were detected by incubation with fluorescein isothiocyanate–avidin (Vector Laboratories) diluted to 50 μg/ml in 1% bovine serum albumin, 4X sterile saline citrate, and 0.1% Tween 20 polysorbate preparation at 37° C for 30 minutes. After washing three times for 5 minutes in 4X sterile saline citrate, 0.1% Tween 20 polysorbate preparation at 42° C, slides were mounted with glycerol containing propidium iodide as counterstain and 1,4-diazabicyclo(2.2.2)octane (DABCO, Sigma Chemical Co., St. Louis) to reduce photobleaching. The total time required for preembryo biopsy, fixation, and in situ hybridization of blastomeres is approximately 48 hours.

Results

The embryo biopsy technique described is pictorially represented in Fig. 1, *panels A to E*. A slit was made in the zona pellucida of mouse preembryos at the four-cell stage with a holding pipette and a fine sharpened microneedle (Fig. 1, *panel A*). After the slit was made a biopsy pipette was inserted into the slit (Fig. 1, *panel B*) and gentle suction was applied. Gentle manipulation was used to tease the blastomere from the surrounding blastomeres and through the zona slit (Fig. 1, *panels C and D*). Finally the blastomere and the embryo were released into the media (Fig. 1, *panel E*). The embryo was passed serially through fresh media and cultured under oil at 37° C in 5% carbon dioxide. Embryos manipulated and cultured in this fashion continued to develop and form blastocysts in the majority of cases (Fig. 1, *panel F*).

A series of embryo biopsy experiments are summarized in Table I. The majority of embryos (84%) cultured in our system proceeded to the blastocyst stage. The manipulations required for partial zona dissection did not inhibit embryo growth inasmuch as approximately 90% of embryos treated in this fashion proceeded to blastocyst. The additional steps required to remove a blastomere had a minimal negative impact on the ability of preembryos to develop into blastocysts as 74% continued to divide. Blastocysts from preembryos that underwent biopsy were morphologically indistinguishable from control blastocysts except that the slit in the zona was sometimes apparent. Occasionally the total size of the blastocyst was somewhat smaller with preembryos from which biopsy specimens were obtained but this was not a consistent finding. Later embryo biopsy experiments were performed with a modified biopsy medium that contained bovine serum albumin (0.2%) and sucrose (0.1 mol/L). A series of 31 biopsies performed in this media resulted in an 84% blastocyst rate.

Table I. Embryo biopsy experiments, control experiments (no manipulation), and partial zona dissection only experiments

	Number to blastocyst	*Number tested*	*%*
Control	4	5	
	16	20	
	15	17	
	5	10	
	14	14	
	8	8	
TOTAL	62	74	84
PZD only	12	13	
	9	10	
	9	10	
TOTAL	30	33	90
Embryo biopsy	11	11	
	8	8	
	9	9	
	7	7	
	3	11	
	6	6	
	6	10	
	2	6	
	4	5	
	3	5	
	3	4	
	10	17	
	7	9	
	10	13	
	8	9	
	7	9	
TOTAL	104	139	75

PDZ, Partial zona dissection. Embryos were placed in biopsy media (calcium- and magnesium-free phosphate-buffered saline solution with 0.1% EDTA) and partial zona dissection was performed by the method of Malter and Cohen. Embryos from which biopsies had been obtained had a biopsy pipette placed inside the zona and a blastomere was removed (see Fig. 1, panels A to F).

Fig. 2, *panel A*, shows a metaphase spread of mouse chromosome that had been subjected to in situ hybridization with a probe that hybridizes to the sodium and potassium adenosine triphosphatase gene on chromosome 3. This probe had been nick translated in the presence of biotinylated dUTP, which specifically incorporated biotin into the probe. After in situ hybridization with this probe fluoresceine isothiocyanate–linked to avidin was incubated with the slide allowing the specific avidin-biotin interaction to label the DNA sequence of interest with a fluorescent probe. Signal was then visualized with a laser-scanning confocal microscope and an image was constructed by an image processor with data recorded by a photomultiplier detector. The image obtained shows signal on both chromatids of each copy of chromosome 3 (four total signals). Fig. 2, *panel B* shows the same probe hybridized to a blastomere obtained by preembryo manipulation. Two signal domains are noted in this interphase nu-

cleus. Specifically this cell is in the G2 phase in which the synthesis phase has duplicated the chromatids, thus each signal domain has a bilobed configuration. Comparison of metaphase chromosomes with interphase nuclei shows the same signal domains.

Fig. 2, *panel C*, shows a blastomere that has been hybridized with a probe that specifically labels the centromeric portion of the X chromosome. There are two clear signals consistent with a female karyotype. Fig. 2, *panel D*, shows a blastomere hybridized with the X probe that happens to be in metaphase. There is one clear signal consistent with a male karyotype. Even though this cell is in metaphase, in situ hybridization allows one to analyze the DNA for the signal of interest. Finally, Fig. 2, *panel E*, shows a human male metaphase spread hybridized with a chromosome Y–specific probe. One clear signal domain is apparent. In situ hybridization to a blastomere from a human polyspermic embryo is shown in Fig. 2, *panel F*. Two clear major signal domains are noted. Two other cells from this same preembryo had two clear signals as well (data not shown) and presumably this represents an XYY karyotype.

Comment

The revolution in molecular genetics will provide medical science with sophisticated, more sensitive methods for prenatal genetic diagnosis. Assisted reproductive technology has provided access to the human preimplantation embryo. The combination of these two disciplines will allow genetic diagnoses before implantation. The extension of prenatal diagnosis to the preimplantation period will enable fertile patients at genetic risk who would not otherwise opt for abortion to use assisted reproductive techniques to select only those embryos that are not at risk of carrying genetic diseases. The success of this technology as a medical diagnostic discipline requires the development of techniques that will reliably and specifically predict genetic outcome. Polymerase chain reaction has enabled one to specifically amplify DNA sequences of interest over one-millionfold. This technique allows the diagnosis of gene-specific disorders such as cystic fibrosis, sickle cell anemia, Duchenne-type muscular dystrophy, and others. Polymerase chain reaction is limited by the ability of small fragments of contaminating DNA sequences to interfere with a reliable diagnosis. Some of these concerns have been overcome by the development of techniques to prevent this problem. Polymerase chain reaction will not allow one to make a diagnosis in which numeric chromosome analysis is concerned. Karyotype analysis of single cells is not practical because it is limited to metaphase and requires a large number of cells. In situ hybridization is the only technique that will permit analysis of chromosome number in the single cell or small numbers of cells. The development of the capability to detect a number of chromosomes simultaneously (e.g., 13, 16, 18, 21, X, and Y) with different fluorescent-labeled probes is being pursued. This article is the first publication of which we are aware that shows in situ hybridization to blastomeres obtained from embryo micromanipulation. Although the techniques need further refinement before becoming clinically applicable this article shows the potential for the use of in situ hybridization as a tool for preimplantation diagnosis.

Embryo biopsy techniques are being pursued by a number of investigators. Most involve the puncture of the zona pellucida by a sharpened pipette of fine caliber and removal of a blastomere. A critical factor in successful embryo biopsy is the media in which the procedure takes place. We used a phosphate-buffered saline solution that is calcium- and magnesium-free. In addition, a small amount of chelating agent is also used. Both promote the disaggregation of blastomeres. Later experiments were done in the presence of sucrose and bovine serum albumin, which contributed to the ability to obtain an intact blastomere by shrinking it with sucrose and preventing its adherence to the pipettes with albumin. The use of a fine microneedle to make a controlled slit in the zona (partial zona dissection) allows the entry of the biopsy pipette into the preembryo with minimal trauma to the blastomeres. Although this technique requires one to change pipettes to complete the procedure the viability of the preembryo after manipulation is as good as some investigators and better than others. This method of embryo biopsy offers an alternative to methods previously published.

All studies to date that use preembryo biopsy analyzed blastomeres by polymerase chain reaction. Whereas polymerase chain reaction is efficient and reliable it is unable to detect aneuploidy and is hampered by contamination. Visualization of the nuclear material directly in the nucleus allows one to perform numeric chromosome analysis without the problem of contamination. At present our method is not efficient enough to be used clinically. Specifically, the ability to retain an intact blastomere on a glass slide through the in situ hybridization procedure is an inefficient process with $<25\%$ success. Focused efforts to alleviate this problem are under investigation.

In this study, in situ hybridization with various gene probes indicates that even one blastomere from which a biopsy specimen has been obtained may be sufficient to localize the gene. In our study, the adenosine triphosphatase gene on chromosome 3 and the X chromosome repeat probe were localized. An important feature of this procedure is that genes and chromosomes can be localized in interphase nuclei and metaphase chromosomes. In the future it will be possible to localize various other genes and chromosomes of interest

as new probes become available. Human repeat sequences and human chromosome libraries, which are chromosome-specific, are currently available for most chromosomes thus numeric chromosome analysis is a potential clinical application of this technique. Therefore coupling of preembryo biopsy and in situ hybridization may be an important dimension in the development of new procedures for prevention of hereditary disease.

We thank Dr. Maurice J. Mahoney for helpful comments and Drs. Christine Disteche and David Housman for providing probe sets. We also thank Jacques Cohen, PhD, and Henry Malter for teaching us partial zona dissection and Julia Logan, Lorrie Bowen, Joan Menninger, and M.Y. Chang, MD, for technical assistance. Finally, the Carl Zeiss Company generously provided us with equipment and technical support.

REFERENCES

1. Saiki RK, Gelfand DH, Stoffel S, et al. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988;239:487-91.
2. Lichter PL, Cremer T, Borden J, Manuelidis L, Ward DC. Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 1988;80:224-34.
3. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high sensitivity fluorescence hybridization. Proc Natl Acad Sci USA 1986;83:2934-8.
4. Gardner RL, Edwards RG. Control of sex ratio at full term in the rabbit by transferring sexed blastocysts. Nature 1968;218:346-8.
5. Singh EL, Hare WCD. The feasibility of sexing bovine morula stage embryos prior to embryo transfer. Theriogenology, 1980;14:421-7.
6. Picard L, King WA, Betteridge KJ. Production of sexed calves from frozen thawed embryos. Vet Rec 1985,117: 603-8.
7. Leonard M, Kirszenbaum M, Cotinot C, et al. Sexing bovine embryos using Y chromosome specific DNA probes. Theriogenology 1987;27:248.
8. Monk M, Handyside A, Hardy K, Whittingham D. Preimplantation diagnosis of deficiency of hypoxanthine phosphoribosyl transferase in a mouse model for Lesch-Nyhan Syndrome. Lancet 1987;2:423-5.
9. Summers P, Campbell JM, Miller MW. Normal in vivo development of marmoset monkey embryos after trophectoderm biopsy. Hum Reprod 1988;3:389-93.
10. West JD, Gosden JR, Angell RR, et al. Sexing the human preimplantation embryo by DNA:DNA in situ hybridization. Lancet 1987;2:1345-7.
11. Handyside AH, Penketh RJA, Winston RML, Pattinson JK, Delhanty JDA, Tuddenham EGD. Biopsy of human preimplantation embryos and sexing by DNA amplification. Lancet 1989;1:347-9.
12. Holding C, Monk M. Diagnosis of beta-thalassaemia by DNA amplification in single blastomeres from mouse preimplantation embryos. Lancet 1989;2:532-5.
13. Wilton LJ, Shaw JM, Trounsen AO. Successful single-cell biopsy and cryopreservation of preimplantation mouse embryos. Fertil Steril 51:513-7.
14. Wilton LJ, Trounsen AO. Biopsy of preimplantation mouse embryos: development of micromanipulated embryos and proliferation of single blastomeres in vitro. Biol Reprod 1989;40:145-52.
15. Malter H, Cohen J. Partial zona dissection of the human oocyte: a nontraumatic method using micromanipulation to assist zona pellucida penetration. Fertil Steril 1989; 51:139-48.
16. Disteche CM, Tantravahi U, Gandy S, Eisenhard M, Adler D, Kunkel LM. Isolation and characterization of two repetitive DNA fragments located near the centromere of the mouse X chromosome. Cytogenet Cell Genet 1985; 39:262-8.
17. Kent RB, Fallows DA, Geissler E, et al. Genes encoding the alpha and beta subunits of sodium/potassium ATPase are located on three different chromosomes in the mouse. PNAS 1984,84:5369-73.
18. Burns J, Chan V, Jonasson JA, Fleming KA, Taylor S, McGee JOD. Sensitive system for visualising biotinylated DNA probes hybridized in situ: rapid sex determination of intact cells. J Clin Pathol 1985;38:1085-92.

Factors affecting embryo implantation after human in vitro fertilization: A hypothesis

Richard J. Paulson, MD, Mark V. Sauer, MD, and Rogerio A. Lobo, MD

Los Angeles, California

In the clinical practice of human in vitro fertilization, pregnancy is dependent on embryo implantation. Pregnancy is a function of the number of embryos transferred, with multiple embryos resulting in a higher likelihood of pregnancy. We formulated a mathematic model of embryo implantation. This model describes embryo implantation as dependent on three factors—transfer efficiency, embryo quality, and endometrial receptivity. Application of existing embryo implantation data to this model allows the calculation of the approximate value of each of these factors. On the basis of historic data, data obtained from our in vitro fertilization program, and these theoretic considerations, it is our hypothesis that (1) there is an inherent inefficiency associated with the mechanical transfer of embryos into the uterine cavity, which limits the maximal embryo implantation rate; (2) the quality of embryos produced by controlled ovarian hyperstimulation, follicle aspiration, and in vitro fertilization is very high and approaches that of embryos produced in natural cycles in vivo; and (3) endometrial receptivity is markedly diminished in stimulated cycles and is the current rate-limiting step of pregnancy success of in vitro fertilization. (AM J OBSTET GYNECOL 1990;163:2020-23.)

Key words: Embryo implantation, in vitro fertilization, unstimulated in vitro fertilization, endometrial receptivity, oocyte donation

In the clinical practice of human in vitro fertilization (IVF) the majority of pregnancy failures occurs after embryo transfer. Whereas the majority of patients achieve oocyte recovery, fertilization, and cleavage of embryos, only a small proportion of transferred embryos actually implant and result in viable pregnancies. Thus pregnancy after IVF and embryo transfer is dependent on embryo implantation. Furthermore, the pregnancy rate is known to increase when multiple embryos are transferred.[1]

It has been suggested that in the first approximation, embryo implantation can be treated as an independent event.[2] When multiple embryos are transferred, the implantation of one embryo neither inhibits nor enhances the implantation of any of the other embryos. This assumption leads to the equation:

$$P = 1 - (1 - EI)^n$$

where P is pregnancy, EI is the likelihood of implantation of an individual embryo, and n is the number of embryos transferred.[2]

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Southern California School of Medicine, and California Reproductive Health Institute, California Medical Center.

Presented at the Thirty-seventh Annual Meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 21 to 24, 1990.

Reprint requests: Richard J. Paulson, MD, Women's Hospital, Room L-1022, 1240 N. Mission Road, Los Angeles, CA 90033.

6/6/24778

We developed a mathematic model for embryo implantation based on the tenet that embryo implantation is an independent event and we described embryo implantation as consisting of three separate components with individual values that vary under specific clinical conditions.

The purpose of this article is to present our mathematic model of factors that affect embryo implantation, to analyze available embryo implantation data in an effort to test and validate this model, and to determine the relative contribution of each of the putative factors under specific clinical conditions.

Material and methods

To develop our hypothesis of factors that affect embryo implantation, we followed three steps. First, we considered previous mathematic formulations and expounded on them. Second, we used embryo implantation data from published sources to modify our model and to help express a maximal attainable rate for embryo implantation. Finally, we examined embryo implantation data from three groups of patients in our own IVF program to test our hypothesis and to assign specific values to the components of the embryo implantation equation.

Hypothesis. The concepts of embryo quality and endometrial receptivity have been used to describe pregnancy success[2, 3]:

Embryo implantation = Embryo quality × endometrial receptivity

Whereas ideal embryo quality and endometrial receptivity may inevitably lead to embryo implantation in vivo, we do not believe that the same will occur when embryos undergo extracorporeal manipulation and mechanical transfer to the uterus. Therefore we chose to group the factors that affect embryo implantation into three general categories including the previously introduced concepts of embryo quality and endometrial receptivity and add a third term, that of *transfer efficiency*. The latter term quantifies the failure of embryo implantation resulting from the embryo transfer process itself. Thus in the first formulation we have:

$$\text{Embryo implantation} = \text{Embryo quality} \times \text{endometrial receptivity} \times \text{transfer efficiency}$$

Transfer efficiency. Transfer efficiency is a term that may be different under different circumstances. It may vary with transfer catheters, the position of the patient, the clinician who performs the transfer, and the different routes of embryo transfer, such as transcervical versus the fallopian tubes. Therefore in estimating this value we chose to focus on implantations after embryo transfer by the transcervical route because this is the most commonly used technique in both our program and in most other institutions. Because transfer efficiency is equal to embryo implantation when both embryo quality and endometrial receptivity are optimal, we thought the best approximation to this value would be obtained by examination of the implantation rates after the synchronous transfer in natural cycles of in vivo fertilized blastocysts. We are aware of two published series in human beings that address this issue.[4, 5] Buster et al.[4] reported implantations in two of four blastocysts transferred. Formigli et al.[5] reported implantations in four of six expanded blastocysts transferred and in six of 11 embryos transferred at the morula stage or thereafter, suggesting a range of 50% to 67% for transfer efficiency in human patients. In animals, reported embryo implantation rates after transcervical transfer of in vivo fertilized blastocysts obtained by uterine flushing are in the range of 50% to 75%.[6] Therefore as a best estimate we believe that with experienced clinicians the approximate value of transfer efficiency for transcervical embryo transfer is 60%.

After updating our formula we now have:

$$\text{Embryo implantation} = \text{Transfer efficiency} \times \text{embryo quality} \times \text{endometrial receptivity}$$

$$\text{Transfer efficiency} = 0.6$$

$$\text{Embryo implantation} = 0.60 \times \text{embryo quality} \times \text{endometrial receptivity}$$

Embryo quality. Because an in vivo fertilized blastocyst is considered to have an embryo quality value of 1, and not all 48-hour embryos become blastocysts, a correction term is needed to account for the clinical practice of transferring embryos 48 hours after follicle aspiration. This term is the percentage of 2-day embryos that progress to the blastocyst stage and provides the maximum quality value that can be assigned to an embryo after 48 hours of development.

In the most commonly used animal model, the mouse, in vivo fertilized embryos collected at the 1- or 2-cell stage progress to blastocyst in about 75% of cases.[7] In human beings, IVF embryo freezing studies reported about a 25% progression to blastocyst, although this number would probably be higher if all embryos were cultured rather than only those that remain after fresh embryo transfer.[8] On the basis of these admittedly scant data, we chose to estimate the percent progression to blastocyst of 48-hour old human embryos at 60%.

Restatement of our original formula:

$$\text{Embryo implantation} = \text{Transfer efficiency} \times \text{embryo quality} \times \text{endometrial receptivity}$$

$$\text{Embryo quality (corrected)} = 0.6 \times \text{embryo quality}$$

$$\text{(Transfer efficiency} = 60\%\text{, as stated in the preceding section)}$$

With these estimates,

$$\text{Embryo implantation} = 0.36 \times \text{embryo quality} \times \text{endometrial receptivity}$$

and thus when embryo quality and endometrial receptivity are optimized,

$$\text{Embryo implantation} = 36\%$$

With this theoretic formulation, we now analyzed embryo implantation data from our IVF program in an effort to test and apply our hypothesis.

Patient selection. Embryo implantation data was obtained in a retrospective fashion from three groups of patients in our program.[9-11] Our nonanonymous donor oocyte program provides a unique model of embryo implantation because it is conducted in a manner identical to standard IVF.[9] In particular, ovarian stimulation and follicle aspiration regimens are identical and all resultant embryos are available for transfer and thus provide embryos of quality similar to that of standard IVF. Embryos are transferred to the uteri of agonadal recipients receiving exogenous estradiol and progesterone whose endometrial histology is synchronous as proved by biopsy in a prior replacement cycle. A total of 18 cycles were conducted between January 1988 and June 1989 with donors who were 35 years old or younger and who had conceived at least once previously. From our standard IVF program, we selected for analysis cycles that we thought would result in embryo quality identical to that of the donor cycles.[10] We considered only patients who were 35 years old or younger, who

Table I. Embryo implantation data

	Embryos transferred		*Implantations**		*Clinical pregnancies*	
	No.	*% per cycle*	*No.*	*%*	*No.*	*%*
dIVF† (n = 18)	75	4.2	26	35	12	67‡
sIVF§ (n = 54)	225	4.2	24	11‡	21	39
uIVF‖ (n = 24)	24	1.0	5	21	5	21

*Total number of gestational sacs observed by transvaginal ultrasonographic examination in each group and the percentage of likelihood of implantation of an individual embryo.

†Donor oocyte IVF group.[9, 10]

‡$p < 0.05$ vs other groups.

§Standarad IVF group; patients were matched to donors in the donor oocyte IVF group.[10]

‖Unstimulated cycles triggered with hCG. Only embryos derived from successfully aspirated dominant follicles were considered.[11]

had conceived at least once previously, and in whom pelvic factor was the sole or predominant cause of infertility. A total of 54 such cycles were conducted during the same 18-month period. These data were previously reported in detail.[10] The third group consisted of patients undergoing embryo transfer in unstimulated IVF cycles during January 1988 to December 1989. The unstimulated IVF protocol was previously described.[11, 12] Briefly, it is offered to normally ovulatory patients with pelvic factor whose follicular development in a spontaneous cycle is monitored with serial ultrasonographic examination. Human chorionic gonadotropin (hCG) is administered when the follicle reaches maturity criteria. In this analysis, we considered only the 24 cycles in which embryos that resulted from successfully aspirated dominant follicles were transferred.

Results

Table I depicts the total number of embryos transferred, the total number of implantations, the individual embryo implantation rate, and the clinical pregnancy rate in the donor oocyte, standard, and unstimulated IVF groups.[9-11] As a result of the matching between the donor and standard IVF groups, a similar number of embryos of similar morphologic quality were obtained and transferred in each of these cycles.[10] In the donor group, 75 total embryos were transferred and 26 implanted, for an embryo implantation rate of 35% and a clinical pregnancy rate of 67%. In the standard IVF group, 225 embryos were transferred and 24 of these were implanted for an embryo implantation rate of 11% and a clinical pregnancy rate of 39%. These differences are statistically significant ($p < 0.05$). In the unstimulated group, 24 transferred embryos resulted in five implantations or a 21% implantation rate, which was significantly higher than that of the stimulated cycles ($p < 0.05$). (Clinical pregnancy rates are presented for completeness but are not included in the implantation analysis.)

Comment

Values for embryo quality and endometrial receptivity can now be approximated. In the donor IVF group:

$$\text{Embryo implantation} = 0.35$$
$$\text{Embryo implantation} = 0.36 \times \text{embryo quality} \times \text{endometrial receptivity}$$

implying that

$$\text{Embryo quality} \sim 1$$
$$\text{Embryo receptivity} \sim 1$$

That endometrial recptivity is optimal is not surprising because the endometrial histology had been optimized. However, if embryo quality is also optimal, this means that current stimulation regimens are capable of producing embryos of a quality that is, at least among those embryos selected for transfer, comparable to that of embryos produced in vivo during natural cycles.

In the standard IVF group:

$$\text{Embryo implantation} = 0.11$$
$$\text{Embryo implantation} = 0.36 \times \text{embryo quality} \times \text{endometrial receptivity}$$

thus

$$\text{Embryo quality} \times \text{endometrial receptivity} \sim 0.33$$

However, by design:

$$\text{Embryo quality (standard IVF)} = \text{Embryo quality (donor IVF)} = 1$$

implying that

$$\text{Embryo receptivity (standard IVF)} = 0.33$$

This difference in endometrial receptivity between standard IVF and donor IVF cycles implies that in stimulated cycles, endometrial receptivity is the principal limiting factor of embryo implantation[10] and that as many as two thirds of transferred embryos capable of implanting fail to do so as a result of decreased endometrial receptivity.

In the unstimulated IVF group:

$$\text{Embryo implantation} = 21\%$$
$$\text{Embryo implantation} = 0.36 \times \text{embryo quality} \times \text{endometrial receptivity}$$

thus

$$\text{Embryo quality} \times \text{endometrial receptivity} = 0.60$$

There is no clear way to assign relative value to embryo quality and endometrial receptivity in this case. However, because steroid levels and their patterns of change in these cycles are essentially natural, it seems

reasonable to assume that endometrial receptivity is uncompromised. If that is true, then

$$\text{Embryo quality} = 0.60$$

Because hCG is administered in these cycles before the onset of the luteinizing hormone surge, this diminished embryo quality value may be explained as reflective of incorrect timing of hCG administration, with resultant diminished oocyte quality.

Our formula and the derived approximate values for transfer efficiency, embryo quality, and endometrial receptivity are not to be taken literally. Rather, it is our intent to attempt to explain the differences in embryo implantation rates that we observed in our program and in the reports of others. We believe that the concept of transfer efficiency is an important addition to the previously introduced embryo quality and endometrial receptivity and may help to explain some of the differences in implantation rates observed under different clinical circumstances and help to define a maximal embryo implantation rate. We also tried to extrapolate from available data the approximate value of the maximal embryo implantation rate that can be expected with current techniques. We estimated this value at 36%. This number matches closely the embryo implantation rates observed in our donor oocyte IVF program. This observation led us to the conclusion that selected embryos obtained from fertilization in vitro of oocytes obtained from hyperstimulated cycles are of a quality that approximates that of in vivo fertilized embryos in natural cycles. Under the scrutiny of time and as additional data become available, the approximate values in this article will likely have to be modified. We present them as a first approximation and as a stimulus to further exploration of this topic.

REFERENCES

1. In vitro fertilization/embryo transfer in the United States: 1988 results from the National IVF-ET registry. Fertil Steril 1990;53:13.
2. Walters DE, Edwards RG, Meistrich ML. A statistical evaluation of implantation after replacing one or more human embryos. J Reprod Fertil 1985;74:557.
3. Rogers PAW, Milne BJ, Trounson AO. A model to show human uterine receptivity and embryo viability following ovarian stimulation for in vitro fertilization. J In Vitro Fert Embryo Transfer 1986;3:93.
4. Buster JE, Bustillo M, Thorneycroft IH, et al. Nonsurgical transfer of in vivo fertilized donated ova to five infertile women: report of two pregnancies. Lancet 1983;2:223.
5. Formigli L, Formigli G, Roccio C. Donation of fertilized uterine ova to infertile women. Fertil Steril 1987;47:162.
6. Anderson GB. Embryo transfer in domestic animals. Adv Vet Sci Comp Med 1983;27:129.
7. Davidson A, Vermesh M, Lobo RA, Paulson RJ. Mouse embryo culture as quality control for human in vitro fertilization: the one versus two-cell model. Fertil Steril 1988; 49:516.
8. Fehilly CB, Cohen J, Simons RF, Fishel SB, Edwards RG. Cryopreservation of cleaving embryos and expanded blastocysts in the human: a comparative study. Fertil Steril 1985;44:638.
9. Sauer MV, Paulson RJ, Macaso TM, Francis-Hernandez M, Lobo RA. Establishment of a nonanonymous donor oocyte program: preliminary experience at the University of Southern California. Fertil Steril 1989;52:433.
10. Paulson RJ, Sauer MV, Lobo RA. Embryo implantation following human in vitro fertilization: importance of endometrial receptivity. Fertil Steril 1990;53:870.
11. Paulson RJ, Sauer MV, Francis MM, Macaso TM, Lobo RA. In vitro fertilization in unstimulated cycles [Abstract 437]. In: Proceedings of the thirty-seventh annual meeting of the Society for Gynecologic Investigation, St. Louis, March 21-24, 1990.
12. Paulson RJ, Sauer MV, Lobo RA. In vitro fertilization in unstimulated cycles: a new application. Fertil Steril 1989; 51:1059.



Prediction of residual trophoblastic tissue in first-trimester abortions: The role of transvaginal Doppler ultrasonography

To the Editors: Schiff and colleagues[3] excellent article on the prediction of residual trophoblast (Schiff E, Ben-Baruch G, Moran O, et al. Prediction of residual trophoblastic tissue in first-trimester abortions and low levels of human chorionic gonadotropin β-subunit. AM J OBSTET GYNECOL 1990;162:797-801) illustrates the importance of a clinical scoring system in the management of first-trimester abortions. I would like to suggest transvaginal pulsed Doppler ultrasonography as an additional useful diagnostic tool for the diagnosis of residual trophoblastic tissue in these patients.

Transvaginal pulsed Doppler ultrasonography has a great advantage potential over routine ultrasonography in tissue characterization because it can display functional flow in addition to structure. Pulsed-wave Doppler ultrasonography allows direct and precise visualization of the sample volume where the flow is to be studied. When Doppler ultrasonography is mounted on a high-frequency transvaginal transducer (6.5 to 7 MHz) it is possible to study flow even in very small structures. In normal and abnormal pregnancies the

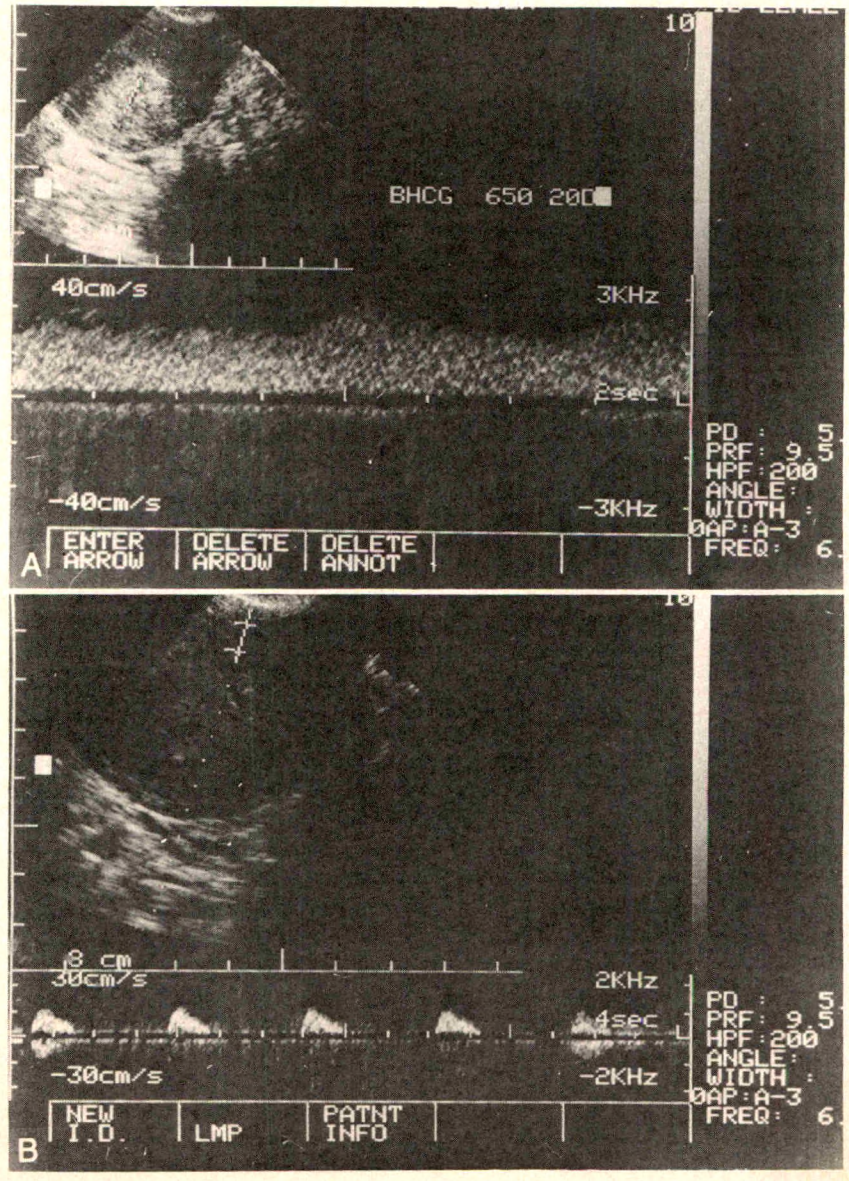


Fig. 1. Ultrasonographic duplex examination of the uterus. **A,** Doppler gate positioned into echogenic tissue. Low-impedance signal appearing during diastole is characteristic of trophoblastic flow. **B,** Intramyometrial Doppler gate shows absence of diastolic flow, consistent with higher distal vascular impedance that is characteristic of nonpregnant arcuate artery.

peritrophoblastic area, which is composed of lacunar (intervillous) spaces devoid of arteriolar muscle in their walls, generates a low-impedance signal during diastole because of its low peripheral resistance.[1] Because histologic examination of the residual trophoblastic tissue shows villi and spaces distended with blood, it is possible to obtain low-impedance signal that is characteristic of placental flow.

The following case illustrates the feasibility of transvaginal pulsed Doppler ultrasonography in assessment of residual trophoblast in first-trimester abortion. Twenty days after curettage for first-trimester abortion the patient had persistent vaginal bleeding. Human chorionic gonadotropin β-subunit was 650 mIU/ml. Transvaginal ultrasonography showed intracavitary echogenic material that might be blood clots or residual trophoblast. Pulsed Doppler investigation of this area clearly depicated low-impedance diastolic signal compatible with trophoblast (Fig. 1). Trophoblastic tissue was obtained by repeated curettage.

Incorporation of transvaginal pulsed Doppler ultrasonography into the proposed scoring system as described by Schiff et al. might be beneficial in improving its diagnostic sensitivity thus reducing unnecessary surgical interventions in the management of first-trimester abortions. Therefore I recommend routine use of transvaginal pulsed Doppler ultrasonography in patients suspected of residual trophoblastic tissue.

Achiron Reuwen, MD

Department of Obstetrics and Gynecology, Shaare-Zedek Medical Center, Jerusalem, Israel 91031

REFERENCE

1. Schaaps JP, Soyeur D. Pulsed Doppler on a vaginal probe. J Ultrasound Med 1989;8:315-20.

Suprapubic or vaginal procedure

To the Editors: We refer to Dr. Arieh Bergman and collaborators' articles (Bergman A., Ballard CA, Koonings PP. Comparison of three different surgical procedures for genuine stress incontinence: prospective randomized study. Am J Obstet Gynecol 1989;160:1102-6) and (Bergman A, Koonings PP, Ballard CA. Primary stress urinary incontinence and pelvic relaxation: prospective randomized comparison of three different operations. Am J Obstet Gynecol 1989;161:97-101). The conclusion is the indubitable superiority of the retropubic procedure in all stress urinary incontinence cases, with or without significant descensus. Seemingly, with a procedure that has a probability of one failure in ten cases, it would be almost malpractice to choose another method with a failure rate of nearly one to three. There is no unanimity with regard to such a marked difference, nor does it confirm our experience with over 500 incontinence operations. More significantly, we believe that the authors' manner to attain this conclusion is not probatory.

Random selection means no selection at all. In the great variety of stress urinary incontinence clinical manifestations, certain criteria give a fairly good orientation on the probable success of vaginal operations. Marked descensus, good accessibility, and good tissular turgor make the vaginal procedure promising. Random selection implies that some patients have undergone vaginal surgery without the aforementioned conditions. Possibly, most gynecologists would prefer suprapubic operations in these cases, this affecting success and failure rates. Bladder neck stabilization does not reach the same extent in the vaginal procedure as in the suprapubic approach. Yet, this may not be necessary. Other factors may intervene.

The critical Q-tip test angle given in the two articles is 30° to 35°. In large series we observed that the critical angle is not the same for both operations. Angles of 25° generally suffice in vaginal operations but frequently result in failure in the suprapubic method. Several factors may explain this difference, independently of bladder neck stabilization. Two are mentioned: the kinking of the urethra and the diminished rectal pressure-transmission in the suprapubic approach are very convincing in the Burch modification, which builds a fibrous wall from one Cooper's ligament to the other, englobing the posterior vaginal vault. Perhaps there is another factor. With a sudden raise of the intraabdominal pressure there may be an instantaneous engorgement of the periurethral veins acting as a veinous cushion increasing urethral pressure, apparently remaining in the vaginal operation, and, to a high degree, obliterated in suprapubic surgery. This may account for the differences we observed in the effective angles between the vaginal and the suprapubic procedures.

With rigorous selection, we believe there is room left for stress urinary incontinence vaginal corrections. The anatomic superiority of bladder neck stabilizations in the suprapubic procedure does not invariably mean functional superiority.

Jaime A. Cabrera, MD, and Zsigmund Szekely, MD
Clinica San Juan Bosco, Calle 170 No. 8-60, Bogota, Colombia
Jairo A. Ospitia, MD
Clinica Federman, Calle 58A No. 37-10, Bogota, Colombia

Reply

To the Editors: We thank Drs. Cabrera, Ospitia, and Szekely for their interest in our articles. These articles indeed indicate that in our hands the abdominal retropubic procedures resulted in a better cure rate than that of the vaginal operations in women with stress incontinence. However, it is a total misinterpretation of the articles to suggest that the vaginal procedures should not be done. We mention in the articles that some authors such as Beck with anterior colporrhaphy[1] or Leach et al. with modified Pereyra procedure[2] achieved good results. These authors modified the "original" technique, resulting in good cure rates.

Our study was designed as a randomized prospective study and random selection of the patients is a key factor in it. In many retrospective studies, women with marked pelvic relaxation (many of whom had more severe degrees of stress urinary incontinence) underwent vaginal operation whereas many women with mild pelvic relaxation underwent abdominal procedures, making the comparison unfair.

Women with no pelvic relaxation and well-supported pelvic organs usually do not have stress urinary incontinence and the few exceptions who do, should not undergo a procedure for bladder neck suspension.[3] However, we clearly indicated in the first article[1] that all women in this study had some degree of pelvic relaxation and all of them had positive Q-tip test results of ≥35°. With these criteria, we believe that randomizing patients to the various procedures is the most meaningful way to conduct such a study.

The theory about the superiority of the vaginal procedures that enable the periurethral veins to remain engorged, as opposed to the abdominal procedures, is very interesting, but remains to be proven. In the comment section of our articles we state clearly that there is definitely room for the vaginal procedures for stress urinary incontinence. The surgeon's experience and the patient's general condition are very important factors to consider. However, in comparing the anterior colporrhaphy or Pereyra procedure with the Burch operation, the latter in our hands resulted in better cure rate.

Arieh Bergman, MD, Charles A. Ballard, MD, and Paul P. Koonings, MD

Los Angeles County–University of Southern California Medical Center, Women's Hospital, Room L-1022, 1240 N. Mission Road, Los Angeles, CA 90033

REFERENCES

1. Beck PR, McCormick S. Treatment of urinary stress incontinence with anterior colporrhaphy. Obstet Gynecol 1982;59:269.
2. Leach GE, Yip CM, Donovan BJ. Mechanism of continence after modified bladder neck suspension. Urology 1987;29:328.
3. Bergman A, Koonings PP, Ballard CA. Negative Q-tip test as a risk factor for failed incontinence surgery in women. J Reprod Med 1989;34:193.
4. Bergman A, Ballard CA, Koonings PP. Comparison of three different surgical procedures for genuine stress incontinence: prospective randomized study. Am J Obstet Gynecol 1989;160:1102.

Tubal embryo successfully transferred in utero

To the Editors: Until fairly recent times, the multifarious aspects of in vitro fertilization, embryo handling, and gamete intrafallopian transfer many would have considered hardly conceivable. In view of all the developments, it is considered timely to report that in the Gifford Memorial Hospital, Randolph, Vermont, in 1980, a 27-year-old patient with unaccountable infertility and regular 28-day cycles had intercourse around the middle of the month. Approximately 4 weeks later severe pain developed in the region of the left fallopian tube. There was no uterine bleeding and the pregnancy test was positive. Her history and all laboratory test results were normal. Abdominopelvic examination revealed no appreciable uterine enlargement or discernible abnormalities, only severe pain elicited with palpation in the tubal area.

Exploration while the patient was under regional anesthesia revealed a single corpus luteum in the left ovary, some uterine congestion, and on direct palpation of the left tube a small, 4 to 5 mm mass. With careful incision into the tubal lumen an intact embryonic sac was easily enucleated, still completely covered with chorionic villi, even at 40 days, as shown in Fig. 1. It was immediately placed in oxygenated saline solution warmed to body temperature. A segment of infusion tubing was cut, one end slanting and the other attachable to a glass Presto syringe with a large rubber bulb enabling one to aspirate or express as desired. With gentle suction the slanted end of the tubing was passed into the myometrium in the upper, anterior aspect of the uterus until discernible decidual tissue was observed. With the tubing in situ, the embryonic sac was taken up into the glass syringe, which was then attached to the tubing and expressed in utero. Tamponade of the puncture site with a very warm pad controlled any bleeding. The tube was then repaired and the abdomen closed. The pregnancy test remained positive. After normal postoperative and prenatal courses, a normal infant was delivered at term.

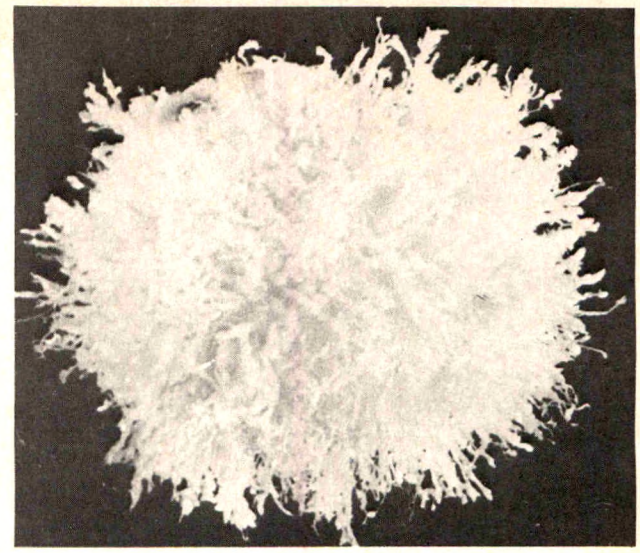


Fig. 1. Human chorionic sac still completely covered with villi at 40 days. (From Rugh R, Shettles LB. From conception to birth: the drama of life's beginnings. New York: Harper & Row, 1971.)

The above was considered the first transfer of a tubal embryo in utero with successful outcome, until recently. Dr. A. P. D. Rosa of São Paulo, Brazil, cites a case he discovered by Dr. C. J. Wallace.[1] The patient underwent a myomectomy and an undiagnosed tubal pregnancy was found. With the myomectomy completed, an "olive size" gestational sac was easily removed and securely placed in utero. After closure of the uterus, the tube, and the abdomen and with uneventful postoperative and prenatal course, a normal infant was delivered at term.

With his case and the one cited, it would appear that with the intact, early embryonic sac still covered with

the full complement of chorionic villi, free of hemorrhage, transfer in utero may prove successful and merits a try, especially in the childless patient.

Landrum B. Shettles, PhD, MD
Womens Hospital, 2025 East Sahara Ave., Las Vegas, NV 89104

REFERENCE

1. Wallace CJ. Transplantations of ectopic pregnancy from fallopian tube in cavity of uterus. Surg Gynecol Obstet 1917;24:578-9.

Use of midforceps applauded

To the Editors: I congratulate Dr. R. A. Bashore on his excellent presentation (A comparison of the morbidity of midforceps and cesarean delivery. AM J OBSTET GYNECOL 1990;162:1428-35.). It is satisfying to see prominent obstetricians doing something to bring clear facts about the advantages of midforceps delivery.

With reference to the 3-hour second stage of labor, I feel better with the classic 2 hours. In special cases of dystocia with good fetal heart tones and epidural anesthesia, the 3 hours are okay. I have seen it longer than that, although contractions every 3 minutes for a longer time bother me because there is no progress and the fetal head receives the trauma.

I am impressed with Dr. Bashore's assessment of future cesarean morbidity. His concerns were: the morbidity associated with repeat cesarean delivery, repeated blood transfusions, and the risk of uterine rupture. To these I would add increased dyspareunia after the age of 50 years and the risk of unusual bleeding when doing a curettage if care is not taken that the curette does not touch the lower anterior cervical segment.

I applaud his guidelines to ensure the prudent use of midforceps. If I were to add my input, I suggest the posterior and transverse position be rotated by the Ramirez technique, which is less traumatic. In dystocia cases, this rotation can be done without anesthesia, the labor is allowed to continue, and later on, if a spontaneous delivery does not occur, you will be doing a forceps delivery at a much lower station and to an anterior position. I suggest that there is a need for a more objective and accurate method to evaluate the presenting part station. The method at present is subjective and inaccurate. On this same point, sometimes I estimate +2, and a few minutes later, I estimate 0 station. We have to be careful with it.

I could not agree more with Dr. Bashore's quest to remove "some of the stigma associated with the use of midforceps without a loss of respect for the procedure."

Jorge O. Escamilla, MD
700 DeSoto Ave., Brooksville, FL 34601

Findings not new

To the Editors: We note with interest the findings of Langer et al. (Langer O, Levy J, Brustman L, Anyaegbunam A, Merkatz R, Divon M. Glycemic control in gestational diabetes mellitis—How tight is tight enough: Small for gestational age versus large for gestational age? AM J OBSTET GYNECOL 1989;161:646-53) who report a correlation between levels of control of maternal glucose and fetal size at birth in mothers diagnosed to have gestational diabetes. They found that when mean maternal blood glucose values are high (>105 mg/dl) there is a significantly increased incidence of large-for-gestational-age infants, whereas in the group with low maternal blood glucose values (mean <86 mg/dl) small-for-gestational-age infants were more likely.

Whereas we are in agreement with the significance of these observations, we write to assert for the benefit of your readers that these findings are not new, and more importantly that hypoglycemia in the whole population of pregnant women (not only those with diabetes) is associated with fetal growth retardation and unfavorable perinatal outcome. Karlsson and Kjellmer reported in 1972[1] of the association between fetal outcome and glycemic control in diabetic mothers, and we have reported regularly from the Mercy Maternity Hospital since the early 1970s of the association between maternal plasma glucose levels and perinatal mortality and morbidity including fetal size and malformations.[2-5]

Our studies were conducted on a pregnant population with oral glucose tolerance routinely tested during the third trimester, not just those patients with criteria that suggested an increased probability of diabetes. Percentile distributions of plasma glucose levels were used to define hypoglycemia and hyperglycemia, and analysis of the obstetric results in 5000 consecutive patients showed that hypoglycemia had a significant association with subnormal estriol excretion, fetal and placental growth retardation, some fetal malformations, and increased perinatal mortality. When there was hyperglycemia placental growth was stimulated, there as an increased incidence of malformations, and perinatal mortality was increased. We found that hyperglycemia of itself was not associated with an increased incidence of large-for-dates infants, although that observation was confirmed in those patients whose glucose tolerance tests showed defined gestational diabetes. We hypothesized that hypoglycemia is a causal factor in fetal growth retardation and death by restriction of basic substrate necessary for normal fetal growth. We proposed that fetal growth retardation in hypoglycemic mothers may be preventable by administering nutrients to mothers either orally or intravenously.[7]

As pleased as we are that researchers continue to publish information that substantiates our earlier findings, we would be grateful that when authors seek to show originality in their research that they would at least acknowledge those publications that first reported the significance of these observations.

David A. Abell, MGO, and Normal A. Beischer, MD
University of Melbourne Department of Obstetrics and Gynaecology, Mercy Maternity Hospital, Clarendon Street, East Melbourne, Victoria, Australia 3002

REFERENCES

1. Karlsson K, Kjellmer I. The outcome of diabetic pregnancies in relation to the mother's blood sugar level. AM J OBSTET and GYNECOL 1972;112:213-20.
2. Macafee CAJ, Beischer NA, Willis MM, Wood C. The correlation of fetal, placental and maternal weight with glucose tolerance. Aust NZ J Obstet Gynaecol 1974;14:88-94.
3. Abell DA, Beischer NA. The relationship between maternal glucose tolerance and fetal size at birth. Aust NZ J Obstet Gynaecol 1976;16:1-4.
4. Abell DA, Beischer NA. Evaluation of the 3-hour oral glucose tolerance test in detection of significant hyperglycemia and hypoglycemia in pregnancy. Diabetes 1975;24:874-80.
5. Abell DA, Beischer NA, Wood C. Routine testing for gestational diabetes, pregnancy hypoglycemia, and fetal growth retardation, and results of treatment. J Perinat Med 1976;4:197-212.
6. Beischer NA, O'Sullivan EF. The effect of rest and intravenous infusion of hypertonic dextrose on subnormal estriol excretion in pregnancy. AM J OBSTET GYNECOL 1972;113:771-8.

Reply

To the Editors: We were pleased to note the interest and agreement of both Drs. Abell and Beischer on the significance of our findings of the correlation between levels of glycemic control and fetal size in patients with treated gestational diabetes. However, we are disappointed that they failed to comprehend the uniqueness of the study and its originality.

Previously, we documented the need for verified self-monitoring blood glucose data to ascertain accurate and reliable glucose information. Without this method to obtain glucose data, false conclusions could have been drawn in previous studies on the relationship between glucose level and neonatal morbidity. Our current study is the only published report of which we are aware that shows the association between degree of glycemia and pregnancy outcome with the use of verified glucose information.[1,2]

The importance of glycemic control has been emphasized from the late 1960s. Most authors agree that reasonable blood glucose levels should be obtained but there are different opinions as to the target level. One study from Iowa City shows that the prognosis was better for infants when the control of the maternal diabetes was "good" than when it was "poor," but the study failed to define either level.[3] Thus, to the best of our knowledge, no study before ours correlated the specific thresholds of verified mean blood glucose values throughout pregnancy with fetal size. Our study addressed two significant questions: at what level should maternal glucose be maintained to prevent perinatal morbidity in gestational diabetes? and can "tight" control limit fetal growth?

The references that Drs. Abell and Beischer cited to emphasize their argument are unrelated to the research question addressed in our study. Karlsson et al.[4] primarily studied insulin-dependent patients and not those with gestational diabetes. Their study was retrospective covering a 10-year period (1961 to 1970) before the establishment of self-monitoring of blood glucose levels (1980). They reported the association between blood glucose level and perinatal mortality as well as the relationship between mean blood glucose and congenital anomalies. However, they did not assess whether "tight" control can alter fetal growth.

The studies by Abell and Beischer in the early 1970s never addressed the question of at what level should maternal glucose be maintained to prevent perinatal morbidity in gestational women? Their studies originated from a data base that correlated estriol and pregnancy outcome. They further attempted to find an association between oral glucose tolerance test (diagnostic tool) findings and fetal size. The majority of their population did not have diabetes, and the glucose load was 50 gm, not 100 gm as recommended by the National Diabetic Data Group. Thus their data cannot be compared with other studies of oral glucose tolerance tests. They did not report the actual mean blood glucose level throughout pregnancy in their gestational diabetic subjects. They used a diagnostic test result to predict outcome of pregnancy, ignoring the fact that treatment of the gestational diabetic patient will alter mean blood glucose level. Thus test results could not be a predictor when intervention occurred. Results of the oral glucose tolerance test could be used as a potential predictor of fetal size in nondiabetic subjects (no intervention) as they, and others, have shown.[5-8]

We acknowledged preliminary research that had stimulated our hypotheses but we cannot, in this instance, recognize their research, which never addressed or showed the findings we reported. We all like to see ourselves in the literature but there still needs to be justification for the citation.

Oded Langer, MD

Division of Obstetrics, The University of Texas Health Science Center, SA, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836

REFERENCES

1. Langer O, Mazze R. Diabetes in pregnancy: evaluation of self-monitoring performance and glycemic control with memory-based reflectance. AM J OBSTET GYNECOL 1986; 155:635-7.
2. Langer O. Scientific rationale for management of diabetes in pregnancy: recent approaches with innovative computer-based technology. Diabetes Care 1988;11(suppl 1):67-72.
3. Delaney J, Ptacek J. Three decades of experience with diabetic pregnancies. AM J OBSTET GYNECOL 1970;106:550.
4. Karlsson K. Outcome of diabetic pregnancies in relation to the mother's blood sugar level. AM J OBSTET GYNECOL 1972;112:213-20.
5. Abell DA, Beischer NA. Evaluation of the 3-hour oral glucose tolerance test in detection of significant hyperglycemia and hypoglycemia in pregnancy. Diabetes 1975;24:874-80.
6. Langer O, Damus K, Maiman M, Divon M, Levy J, Bauman W. A link between relative hypoglycemia-hypoinsulinemia during oral glucose tolerance tests and intrauterine growth retardation. AM J OBSTET GYNECOL 1986;155:711-6.
7. Sokol RJ, Kazzi GM, Kalhan SC, Pillay SK. Identifying the pregnancy at risk for intrauterine growth retardation: possible usefulness of the intravenous glucose tolerance test. AM J OBSTET GYNECOL 1983;143:220-3.
8. Khouzami VA, Ginsburg DS, Daikoku NH, Johnson JWC. The glucose tolerance test as a means of identifying intrauterine growth retardation. AM J OBSTET GYNECOL 1981; 139:423-6.

Explanation of mechanism of Hyskon solution reaction needed

To the Editors: Drs. Jedeikin, Olsfanger, and Kessler's article (Disseminated intravascular coagulopathy and adult respiratory distress syndrome: life-threatening complications of hysteroscopy. AM J OBSTET GYNECOL 1990;162:44-5) is one of several reports of this complication. Whether this complication occurs because of an idiosyncratic reaction to or the intravascular injection of an excessive amount of Hyskon solution, or a combination of these causes has not been determined.

What occurred in this case is clear but the authors need to clarify several factors if we are to have a better understanding of the mechanism of this problem. The article implies that the 1200 ml of Hyskon solution injected into the uterine cavity were retained by the patient. If this is so, how did it occur? Passage of large amounts of Hyskon solution through the fallopian tubes is unlikely because of the viscosity of this material. Intravasation without excessive pressure is unlikely because this was not a surgical hysteroscopic procedure in which vessels were opened. Was it caused by distension with excessive pressures and the intravasation of Hyskon solution through ruptured uterine vessels? How was distension accomplished and what were the resultant pressures? What do the authors believe happened?

Franklin D. Loffer, MD
3410 N. 4th Ave., Phoenix, AZ 85013

Safety of 32% dextran 70 for hysteroscopy

To the Editors: In a recent case report (Jedeikin R, Olsfanger D, Kessler I. Disseminated intravascular coagulopathy and adult respiratory distress syndrome: life-threatening complications of hysteroscopy. AM J OBSTET GYNECOL 1990;162:44-5) the authors claim that alarming symptoms observed in a previously healthy young woman after hysteroscopy were caused by disseminated intravascular coagulopathy and adult respiratory distress syndrome as a result of the use of high-molecular-weight dextran (Hyskon) solution. Neither of these claims is supported by the presented findings. In my opinion this report is an example of the uncritical use of Hyskon solution by surgeons not completely familiar with its properties and other such reports that have been published recently.[1]

It is obvious that during hysteroscopy Hyskon solution may pass into the peritoneal cavity and be absorbed, and also that Hyskon solution may directly enter the circulation during hysteroscopic surgery. It is recommended in the U.S. package insert for Hyskon solution that not more than 500 ml should be instilled and with low pressure to avoid undue passage into the abdomen. In the referred case report Hyskon solution was used in a much higher dosage, but the authors fail to draw the conclusion that the symptoms were caused by the overdosage of Hyskon solution, not by the substance used per se.

Hyskon solution appears to be regarded by many users as an inert distension medium, but it contains dextran 70 and has several pharmacologic effects. 100 ml contains 32 gm of dextran capable of binding 20 ml of water per gram if allowed to enter the circulation. In this case 1200 ml Hyskon solution was instilled during hysteroscopy, after which also laparoscopy was performed. This order of events would promote rapid absorption of dextran from the peritoneal cavity during Trendelenburg position. If 25% of the Hyskon solution used was absorbed 96 gm of dextran would enter the circulation, increasing the blood volume by approximately 2000 ml, an increase that may well precipitate pulmonary edema.

Dextran is widely used as a thromboprophylactic agent. It reduces the Factor VIII–von Willebrand complex. The dextran absorbed should not exceed 1.5 gm/kg body weight, or approximately 300 ml of Hyskon solution. In the referred case the amount of dextran may well have been in excess of this amount, causing a temporary hemostatic defect.

Hyskon solution may on rare occasions cause an anaphylactic reaction,[2] but the symptoms reported to not indicate this. Theoretically such reactions could be prevented by the use of hapten inhibition, which has successfully been applied to prevent anaphylactic reactions from dextrans used intravenously,[3] but the rarity of such reactions with Hyskon solution makes such a procedure questionable.

Karl-Gösta Ljungström, MD, PhD
Department of Surgery, Karolinska Institute, Danderyd Hospital, Danderyd, Sweden S-182 88

REFERENCES

1. Leake JF, Murphy AA, Zacur HA. Noncardiogenic pulmonary edema: a complication of operative hysteroscopy. Fertil Steril 1987;48:497-9.
2. Trimbos-Kemper TCM, Veering BT. Anaphylactic shock from intracavitary 32% dextran-70 during hysteroscopy. Fertil Steril 1989;51:1053-4.
3. Ljungström K-G, Renck H, Hedin H, Richter W, Wiholm B-E. Hapten inhibition and dextran anaphylaxis. Anaesthesia 1988;43:729-32.

Reply

To the Editors: Dr. Ljungstrom concurs that the complications we described may occur after hysteroscopy with Hyskon solution, but makes the point that it is not the substance per se but its overdosage that is responsible for the disseminated intravascular coagulopathy and pulmonary edema. There is no argument that the quantity of Hyskon solution administered was excessive; nevertheless Hyskon solution was the preparation used and Hyskon solution caused the problem, albeit an overdose.

In our case report we did not set out to show the exact mechanism of the problem described; however, we believe that because of the large amount of Hyskon solution injected, a large enough quantity was rapidly absorbed into the circulation via the fallopian tubes and the peritoneal cavity. According to Dr. Ljungstrom, absorption of 25% of the Hyskon solution given would increase blood volume by about 2 L, and occurring over

a short period of time could cause pulmonary edema in healthy persons. On the other hand, the excessive amount of Hyskon solution may have contributed to an adverse drug reaction that manifested by disseminated intravascular coagulopathy and adult respiratory distress syndrome. Further research into the pathophysiology of the toxic effects of Hyskon solution is required to clarify this problem.

The purpose of our article was not to discredit the use of Hyskon solution but to emphasize and to describe the dangers that may occur with the over zealous intrauterine injection of Hyskon solution during simple diagnostic hysteroscopy. All drugs have side effects, and awareness of these complications results in a more intelligent and safer use of these drugs.

R. Jedeikin, MD, and D. Olsfanger, MD
Department of Anesthesia and Intensive Care, Meir Hospital, Kfar Saba, Israel

Corrections

In the list of "New Certifications: Subspecialty of Basic," issued by the American Board of Obstetrics and Gynecology, Inc. (AM J OBSTET GYNECOL 1990;162:1645-68), on page 1652, in the right-hand column, the name "Kassebaum, Hedegaard Cheryl Kaye" should have been "Hedegaard, Cheryl Kay Kassebaum."

In the article by Kivikoski et al., entitled "Transabdominal and transvaginal ultrasonography in the diagnosis of ectopic pregnancy: A comparative study" (AM J OBSTET GYNECOL 1990;163:123-9), in Table I on page 125, in the first line under the third column heading, "84%" should have been "64%" in reference to gestational sac findings on transvaginal ultrasonographic examination.

In the article by Owen et al., entitled "Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population" (AM J OBSTET GYNECOL 1990;163:893-9), Fig. 1 on page 874 should have been published as Fig. 2 on page 876, and Fig. 2 on page 876 should have been published as Fig. 1 on page 874. The legends are correct as they appear.

Books received

Appleton & Lange's Review for National Boards. Part I. Second Edition. Robin J. O. Catlin. 299 pages. East Norwalk, Connecticut, 1989, Appleton & Lange. $30.95.

Appleton & Lange's Review for National Boards. Part II. Second Edition. Robin J. O. Catlin. 299 pages. East Norwalk, Connecticut, 1989, Appleton & Lange. $30.95.

Basic Adolescent Gynecology: An Office Guide. Edited by Victor C. Strasburger. 243 pages, illustrated. Baltimore, 1990, Urban & Schwarzenberg. $45.00.

Benign Breast Disease. Edited by J. A. Smallwood and I. Taylor. 197 pages, illustrated. Baltimore, 1990, Urban & Schwarzenberg. $49.50.

Children of Fast-Track Parents: Raising Self-Sufficient and Confident Children in an Achievement-Oriented World. Andree Aelion Brooks. 258 pages. New York, 1990, Penguin Books. $8.95 (soft cover).

Clinical Hypertension. Fifth edition. Norman M. Kaplan. 466 pages, illustrated. Baltimore, 1990, Williams & Wilkins. $62.50.

Conquering Infertility: A Guide for Couples. Steven L. Corson. 258 pages, illustrated. New York, 1990, Prentice Hall Press. $19.95.

The Crying Baby. Sheila Kitzinger. 295 pages. New York, 1990, Penguin Books. $7.95 (soft cover).

Endosonography. Edited by Bruno D. Fornage. 186 pages, illustrated. The Netherlands, 1990, Kluwer Academic Publishers Group. $129.00.

Environmental Causes of Human Birth Defects. T. V. N. Persaud. 182 pages, illustrated. Springfield, Illinois, 1990, Charles C Thomas, Publisher. $34.75.

Effective Care in Pregnancy and Childbirth. Two volume set. Iain Chalmers, Murray Enkin, and Marc J. N. C. Keirse. 1516 pages, illustrated. New York, 1989, Oxford University Press. $400.00.

The Essential Partnership: How Parents and Children Can Meet the Emotional Challenges of Infancy and Childhood. Stanley I. Greenspan and Nancy Thorndike Greenspan. 256 pages. New York, 1990, Penguin Books. $8.95 (soft cover).

Everyday Parenting: The First Five Years. Robin Goldstein. 267 pages. New York, 1990, Penguin Books. $6.95 (soft cover).

Gynaecology: A Practical Approach. Victor Gomel, Malcolm G. Munro, and Timothy C. Rowe. 332 pages, illustrated. Baltimore, 1990, Williams & Wilkins. $29.95.

Handbook of Drug Therapy in Reproductive Endocrinology and Infertility. Edited by Michel E. Rivlin. Boston, 1990, Little Brown & Company. No price listed.

How to Prevent Miscarriage and Other Crises of Pregnancy. Stefan Semchyshyn and Carol Colman. 242 pages. New York, 1990, Macmillan Publishing Company. $17.95.

Looking Beyond the Ivy League: Finding the College That's Right for You. Loren Pope. 270 pages. New York, 1990, Stephen Greene Press. $7.95.

Motherhood in the Old South: Pregnancy, Childbirth, and Infant Rearing. Sally G. McMillen. 237 pages. Baton Rouge, 1990, Louisiana State University Press. $24.95.

New Options for Fertility. Arthur L. Wisot and David R. Meldrum. 288 pages. New York, 1990, Pharos Books. $17.95.

Pediatric and Adolescent Gynecology. Third Edition. S. Jean Herriot Emans and Donald Peter Goldstein. 621 pages, illustrated. Boston, 1990, Little Brown & Company. $22.50.

Pocket Guide to Medications Used in Dermatology. Andrew J. Scheman. 150 pages. Baltimore, 1990, Williams & Wilkins. $14.95.

The Politics of Maternity Care: Services for Childbearing Women in Twentieth-Century Britain. Edited by Jo Garcia, Robert Kilpatrick, and Martin Richards. 346 pages. New York, 1990, Oxford University Press. $55.00.

Roundsmanship '89: A Year Book Guide to Clinical Medicine. Edited by Bruce B. Dan. 243 pages. Chicago, 1989, Year Book Medical Publishers, Inc. $19.95.

Spermatozoa: The Probable Etiology of Gonorrhea, Nonspecific Urethritis and Other Diseases. Donald E. Tyler. 87 pages. Ontario, Oregon, 1990, Discovery Books. $25.00.

When a Baby Dies: Psychotherapy for Pregnancy and Newborn Loss. Irving G. Leon. 230 pages. New Haven, Connecticut, 1990, Yale University Press. $25.00.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

International Symposium on Advances in Contraception and Contragestion, Lausanne, Switzerland, October 2-4, 1991. For further information contact: Dr. S. Kaul, MEDADVICE, P.O. Box 82, CH-4105 Biel-Benken, Switzerland. Fax: (061) 73 16 12; (061) 721 16 12 after April 22, 1991.

Postgraduate Course in Gynecologic and Obstetric Pathology with Clinical Correlation, April 8-12, 1991, Parker House Hotel, Boston, Massachusetts. Fee for course is $650.00 (residents and fellows, $450.00). Address for further information: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, MA 02115

Fourteenth Annual Mid-Winter Symposium in OB/GYN, Scottsdale Hilton Hotel, Scottsdale, Arizona, February 27–March 1, 1991. Co-sponsored by the Phoenix OB/GYN Society and the Department of OB/GYN, Maricopa Medical Center. Contact: The Secretary, Phoenix OB/GYN Society, 511 W. Montebello, Phoenix, AZ 85013. Tel.: (602) 266-6321.

Issues in Obstetrics and Gynecology, February 25–March 1, 1991, The Hyatt of Sarasota, Sarasota, Florida. For further information contact: American Medical Seminars, Inc., Mr. D. Reece Pierce, P.O. Box 6214, Sarasota, FL 34278. Tel.: (813) 388-1766.

3rd Annual Review Course in Obstetrics and Gynaecology, April 30–May 3, 1991, Delta Chelsea Inn, Toronto, Ontario, Canada. AMA category I study credits. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

Common Problems in Obstetric Care, April 3-6, 1991, Westin Maui, Maui, Hawaii. Sponsored by the Department of Obstetrics and Gynecology, Cornell University Medical College–The New York Hospital and The University Of Hawaii Medical Center. For information contact: Colette Carmeris, Course Coordinator, Department of Obstetrics and Gynecology, NYH-CUMC, 525 East 68th St., New York, NY 10021. Tel.: (212) 746-3058.

American Journal of Obstetrics and Gynecology

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*
FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*
E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*
STEVEN G. GABBE, *Associate Editor*
THOMAS J. GARITE, *Associate Editor*
MOON H. KIM, *Associate Editor*
ALBERTO MANETTA, *Associate Editor*

Advisory committee on policy

Garland Anderson	Walter S. Keifer	Frank Miller
William R. Anderson	M. Tucker Laffitte, Jr.	Daniel R. Mishell, Jr.
Mason C. Andrews	Emmet J. Lamb	John C. Morrison
E. Forrest Boyd	Roger A. Lobo	Karl C. Podratz
Robert C. Cefalo	James M. Maharry	Ruth W. Schwartz
Donald R. Coustan	L. Russell Malinak	James R. Scott
A. Jerome Freeland	Douglas J. Marchant	Henry A. Thiede
Norman F. Gant	Arthur M. McCausland	Carol Trumbold
W. Glenn Hurt	James A. Merrill	Ronald V. Wade

Board of corresponding editors

Luigi Carenza, *Rome, Italy*	Nils-Otto Sjöberg, *Malmö, Sweden*
T. K. A. B. Eskes, *Nijmegen, The Netherlands*	Gordon M. Stirrat, *Bristol, England*
Wolfgang Künzel, *Giessen, West Germany*	Claude Sureau, *Paris, France*
Matsuto Mochizuki, *Kobe, Japan*	Carl Wood, *Melbourne, Victoria, Australia*
M. Reuben Peyser, *Tel Aviv, Israel*	Olavi Ylikorkala, *Helsinki, Finland*

Publisher
MOSBY–YEAR BOOK, INC., 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*

Volume 163
July-December, 1990
Mosby–Year Book, Inc.
St. Louis
1990

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY
OBSTETRICAL SOCIETY OF PHILADELPHIA
BROOKLYN GYNECOLOGICAL SOCIETY
ST. LOUIS GYNECOLOGICAL SOCIETY
NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY
THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND
CHICAGO GYNECOLOGICAL SOCIETY
CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY
WASHINGTON GYNECOLOGICAL SOCIETY
PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY
BOSTON OBSTETRICAL SOCIETY
LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY
SEATTLE GYNECOLOGICAL SOCIETY
ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY
KANSAS CITY GYNECOLOGICAL SOCIETY
CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS
NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY
IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY
THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY
MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY
UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY
ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY
ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY
TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY
NEW YORK GYNECOLOGICAL SOCIETY
PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION
BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY
SAN FRANCISCO GYNECOLOGICAL SOCIETY
JACKSON GYNECIC SOCIETY
INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY
THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY
COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY
GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY
CLEVELAND SOCIETY OF OBSTETRICIANS AND GYNECOLOGISTS

Volume 163
Copyright © 1990 by Mosby–Year Book, Inc.
(All rights reserved)

Printed in the United States of America

Index to volume 163

Author index*

A

Abashawl A (*see* Berkus et al). 1990;163:1818-21
Abbate R, Pinto S, Rostagno C, Bruni V, Rosati D, Mariani G. Effects of long-term gestodene-containing oral contraceptive administration on hemostasis. 1990;163:424-9 (Symposium)
Abbitt PL (*see* Hogge et al). 1990;163:1061-5
Abbott TM (*see* Branch et al). 1990;163:210-6
Abdullah MA (*see* Al Kasab et al). 1990;163:37-40
Abell DA, Beischer NA. Findings not new. 1990;163:2027-8 (Letter)
Abramowicz J (*see* Kuhlmann et al). 1990;163:1264-70
Abramowicz JS (*see* Sherer and Abramowicz). 1990;163:1366-7 (Letter)
Abulafia O (*see* Hoegsberg et al). 1990;163:1135-9
Adams MR, Clarkson TB, Shively CA, Parks JS, Kaplan JR. Oral contraceptives, lipoproteins, and atherosclerosis. 1990;163:1388-92 (Symposium)
—— (*see* Jayo et al). 1990;163:614-8
Adamson SL (*see* Morrow et al). 1990;163:1313-20
Ahokas RA (*see* Mabie et al). 1990;163:1861-7
Akkersdijk GJM, Flu PK, Giard RWM, van Lent M, Wallenburg HCS. Malignant leiomyomatosis peritonealis disseminata. 1990;163:591-3
Akl S (*see* Moretti et al). 1990;163:1844-8
—— (*see* Sibai et al). 1990;163:733-8
Aksel S (*see* Alexander and Aksel). 1990;163:1715 (Letter reply)
Albazzaz SJ (*see* Hadi and Albazzaz). 1990;163:700-1 (Letter reply)
Al Bitar I (*see* Al Kasab et al). 1990;163:37-40
Ales KL (*see* August et al). 1990;163:1612-21
Alexander SE, Aksel S. Parameters of hypothalamic aging. 1990;163:1715 (Letter reply)
Alexander WJ (*see* Barbone et al). 1990;163:510-4
Al Kasab SM, Sabag T, Al Zaibag M, Awaad M, Al Bitar I, Halim MA, Abdullah MA, Shahed M, Rajendran V, Sawyer W. β-Adrenergic receptor blockade in the management of pregnant women with mitral stenosis. 1990;163:37-40
Al Zaibag M (*see* Al Kasab et al). 1990;163:37-40
Ambrose PJ (*see* Briggs et al). 1990;163:251-2 (Letter reply)
Ambrose SE (*see* Shyken et al). 1990;163:802-7
Amon E (*see* Sadovsky and Amon). 1990;163:259 (Letter reply)
Amy J-J (*see* Foulon et al). 1990;163:1511-3
Andersen HF, Nugent CE, Wanty SD, Hayashi RH. Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length. 1990;163:859-67
Andersen O (*see* Skouby et al). 1990;163:343-8 (Symposium)
Anderson DS (*see* Dayton et al). 1990;163:63-4
Anderson LJ (*see* Rodis et al). 1990;163:1168-71
Anderson TL (*see* Osteen et al). 1990;163:479-84
Andersson K-E (*see* Skajaa et al). 1990;163:1323-33
Andres RL, Kuyper W, Resnik R, Piacquadio KM, Benirschke K. The association of maternal floor infarction of the placenta with adverse perinatal outcome. 1990;163:935-8
——, Piacquadio KM, Resnik R. A reappraisal of the need for autologous blood donation in the obstetric patient. 1990;163:1551-3
Andriakos P (*see* Esposito et al). 1990;163:572-4
Angelides EM (*see* Esposito et al). 1990;163:572-4
Angioni S (*see* Petraglia et al). 1990;163:1307-12
Ansbacher R (*see* Punch and Ansbacher). 1990;163:114-6
Applebaum-Bowden D (*see* Wild et al). 1990;163:1998-2005
Araneda C (*see* McCoshen et al). 1990;163:1632-40
Arduini D (*see* Rizzo et al). 1990;163:1231-8
Ariel I (*see* Ben-Chetrit et al). 1990;163:1161-3
Ariet M (*see* Resnick and Ariet). 1990;163:269-70 (Letter reply)
Arikan Ü (*see* Durukan et al). 1990;163:594-5
Arnett RM, Jones JS, Horger EO III. Effectiveness of 1% lidocaine dorsal penile nerve block in infant circumcision. 1990;163:1074-80
Aronson MP (*see* Carr et al). 1990;163:719-22
Arrington J (*see* Kuhlmann et al). 1990;163:1264-70
Ashmead GG (*see* Lazebnik et al). 1990;163:78-80
Ashmead JW (*see* Lazebnik et al). 1990;163:78-80
Asrat T, Nageotte MP, Garite TJ, Gocke SE, Dorchester W. Gram stain results from amniocentesis in patients with preterm premature rupture of membranes—comparison of maternal and fetal characteristics. 1990;163:887-9
Assendorp R (*see* Bergink et al). 1990;163:2132-7 (Symposium)
Athanassiadis AP (*see* Romero et al). 1990;163:823-5
Atrash HK (*see* Saftlas et al). 1990;163:460-5
August P, Lenz T, Ales KL, Druzin ML, Edersheim TG, Hutson JM, Müller FB, Laragh JH, Sealey JE. Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia. 1990;163:1612-21
Auld PAM (*see* Lipper et al). 1990;163:146-50
Austin H (*see* Barbone et al). 1990;163:510-4
Avila C (*see* Romero et al). 1990;163:757-61
—— (*see* Romero et al). 1990;163:823-5
—— (*see* Romero et al). 1990;163:968-74
Awaad M (*see* Al Kasab et al). 1990;163:37-40
Ayromlooi J. Conservative management of cervical pregnancy with subsequent fertility. 1990;163:1093-4 (Letter)
Azuma C (*see* Nobunaga et al). 1990;163:634-8

B

Bachus K. Conservative management of cervical pregnancy with subsequent fertility. 1990;163:1094-5 (Letter reply)
Bachus V (*see* McGregor et al). 1990;163:1580-91
Back DJ, Madden S, Orme ML'E. Gastrointestinal metabolism of contraceptive steroids. 1990;163:2138-45 (Symposium)

*July, part 1, 1-272; part 2, 273-446; August, 447-714; September, 715-1108; October, part 1, 1109-1378; part 2, 1379-1420; November, part 1, 1421-1718; part 2, 1719-1770; December, part 1, 1771-2112; part 2, 2113-218.

—— (*see* Orme and Back). 1990;163:2146-52 (Symposium)
Baggish MS. Fluid cooling of artificial sapphire tips of laser. 1990;163:681 (Letter reply)
——. Hyperbaric oxygen therapy for air embolism complicating operative hysteroscopy. 1990;163:681 (Letter reply)
——, Daniell JF. Sapphire probes in obstetric laser surgery. 1990;163:247 (Letter reply)
Bain RP (*see* Ewigman et al). 1990;163:256-7 (Letter)
Baker PN, Pipkin FB, Symonds EM. Changes in platelet angiotensin II binding sites associated with the human menstrual cycle. 1990;163:175-6
Baker SL (*see* Owen et al). 1990;163:868-72
Baker VV (*see* Kutteh et al). 1990;163:1301-6
Baldwin VJ. Twin transfusion syndrome. 1990;163:1376-7 (Letter)
Ball P, Knuppen R. Formation, metabolism, and physiologic importance of catecholestrogens. 1990;163:2163-70 (Symposium)
Ballagh SA (*see* Posner et al). 1990;163:813-8
Ballard CA (*see* Bergman et al). 1990;163:2025-6 (Letter reply)
Bamford OS, Hawkins RL, Blanco CE. Effects of clonidine on breathing movements and electrocortical activity in the fetal lamb. 1990;163:661-8
Bandstra ES (*see* Burkett et al). 1990;163:40-1
Barbera M (*see* Figueroa et al). 1990;163:648-54
Barbieri RL (*see* Friedman et al). 1990;163:1114-9 (Clin. opinion)
Barbone F, Austin H, Louv WC, Alexander WJ. A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis. 1990;163:510-4
Bard H (*see* Tucci and Bard). 1990;163:1646-9
Bardawil WA (*see* Hecht et al). 1990;163:1986-91
Bardeguez A, Chatterjee M, Tepedino M, Sicuranza B. Systemic cystic angiomatosis in pregnancy: a case presentation and review of the literature. 1990;163:42-5
Barglow P (*see* Rizzo et al). 1990;163:1458-64
Barrett JM (*see* Orr et al). 1990;163:1485-9
Barros C (*see* Fuchs et al). 1990;163:1961-7
Bartholomew M (*see* Wild et al). 1990;163:1998-2005
Barton DPJ (*see* Cavanagh et al). 1990;163:1007-15
Barton JR, Sibai BM, Mabie WC, Shanklin DR. Recurrent acute fatty liver of pregnancy. 1990;163:534-8
Baruch GB, Menczer J. Trend of cervical cancer in Jewish women. 1990;163:703-4 (Letter)
Bast RC Jr (*see* Hunter et al). 1990;163:1164-7
—— (*see* Soper et al). 1990;163:1204-9
Basu J, Mikhail MS, Payraudeau PH, Palan PR, Romney SL. Smoking and the antioxidant ascorbic acid: plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women. 1990;163:1948-52
Basu SN (*see* George et al). 1990;163:1203-4
Batra KK (*see* Gillogley et al). 1990;163:1535-42
Baudo F, Caimi TM, Redaelli R, Nosari AM, Mauri M, Leonardi G, deCataldo F. Emergency treatment with recombinant tissue plasminogen activator of pulmonary embolism in a pregnant woman with antithrombin III deficiency. 1990;163:1274-5
Bauer GS (*see* Long et al). 1990;163:1976-81
Bawdon RE (*see* Brown et al). 1990;163:938-43
—— (*see* Harstad et al). 1990;163:912-6
—— (*see* Roe et al). 1990;163:715-8
Baxi L (*see* Berkowitz et al). 1990;163:975-7
Baxi LV (*see* Shyken et al). 1990;163:802-7
Beaird JA (*see* Silver et al). 1990;163:1914-9
Beamer WG (*see* Tennent et al). 1990;163:625-34
Beckmann I (*see* Leiberman et al). 1990;163:1685-8
Beck-Nielsen H, Nielsen OH, Damsbo P, Vaag A, Handberg [oc]A, Henriksen JE. Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system. 1990;163:292-5 (Symposium)
Beecham J (*see* Nicolini et al). 1990;163:1144-6
Béguin F (*see* Dupont et al). 1990;163:1527-8
Behrens O (*see* Fuchs et al). 1990;163:1961-7
Behzadian A (*see* Tho et al). 1990;163:1968-75
Beischer NA (*see* Abell and Beischer). 1990;163:2027-8 (Letter)
Belanger K (*see* Copel et al). 1990;163:751-6
Ben-Baruch G (*see* Schiff et al). 1990;163:1375 (Letter reply)
Ben-Chetrit A, Yagel S, Ariel I, Zacut D, Shimonovitz S, Celnikier-Hochner D. Successful conservative management of primary nonmetastatic cervical choriocarcinoma. 1990;163:1161-3
Benedetti TJ (*see* Easterling and Benedetti). 1990;163:1104-6 (Letter)
Benirschke K (*see* Andres et al). 1990;163:935-8
—— (*see* Gilbert et al). 1990;163:998-9
—— (*see* Moore et al). 1990;163:907-12
Bennett PR, Elder MG, Myatt L. Secretion of phospholipases by bacterial pathogens may initiate preterm labor. 1990;163:241-2 (Letter)
Benrubi GI (*see* Johnston et al). 1990;163:743-7
Benzaquen S, Gagnon R, Hunse C, Foreman F. The intrauterine sound environment of the human fetus during labor. 1990;163:484-90
Benzi G (*see* Rovati et al). 1990;163:1490-2
Beral V (*see* Franks et al). 1990;163:1120-3
Berchuck A (*see* Hunter et al). 1990;163:1164-7
—— (*see* Soper et al). 1990;163:1204-9
Berek JS (*see* Parker and Berek). 1990;163:1574-7
Bergeron C (*see* Ferenczy et al). 1990;163:1271-4
Bergink W, Assendorp R, Kloosterboer L, van Lier W, Voortman G, Qvist I. Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin. 1990;163:2132-7 (Symposium)
Bergman A, Ballard CA, Koonings PP. Suprapubic or vaginal procedure. 1990;163:2025-6 (Letter reply)
Berkowitz K, Baxi L, Fox HE. False-negative syphilis screening: the prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy. 1990;163:975-7
——, LaSala A. Risk factors associated with the increasing prevalence of pneumonia during pregnancy. 1990;163:981-5
——, McCaffrey R. Postpartum osteomyelitis caused by group B streptococcus. 1990;163:1200-1
Berkus MD, Stern MP, Mitchell BD, Abashawl A, Langer O. Relationships between glucose levels and insulin secretion during a glucose challenge test. 1990;163:1818-21
——, ——, ——, Newton ER, Langer O. Does fasting interval affect the glucose challenge test? 1990;163:1812-7
—— (*see* Wright et al). 1990;163:748-50
Bernedes H (*see* Villar et al). 1990;163:151-7
Bernstein GS (*see* Kjos et al). 1990;163:93-8
—— (*see* Kjos et al). 1990;163:1822-7
Beykal Ö (*see* Durukan et al). 1990;163:594-5
Bilezikjian LM (*see* Petraglia et al). 1990;163:1307-12
Biol D (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Black DM (*see* Cauley et al). 1990;163:1438-44
Blackard WG (*see* Nestler et al). 1990;163:1243-6

Blakemore KJ (*see* Shyken et al). 1990;163:802-7
Blamire AM (*see* Johnson et al). 1990;163:601-7
Blanchet P (*see* Villeneuve et al). 1990;163:1641-6
Blanco AN (*see* Riveros et al). 1990;163:704-5 (Letter)
Blanco CE (*see* Bamford et al). 1990;163:661-8
Blaskiewicz RJ. Ethics of new reproductive technologies. 1990;163:1376 (Letter)
Blesing NE, Hambley H. Early hypothyroidism in patients with menorrhagia. 1990;163:697 (Letter)
Blickstein I. Ritodrine infusion and cardiomyopathy. 1990;163:255-6 (Letter reply)
——. Twin-to-twin transfusion syndrome. 1990;163:677 (Letter)
Blumenfeld Z (*see* Bronshstein and Blumenfeld). 1990; 163:268-9 (Letter reply)
Bolan JC (*see* Burke et al). 1990;163:17-8
Bolaños F (*see* Villar et al). 1990;163:151-7
Bonacina E (*see* Curiel et al). 1990;163:538-9
Bonet V (*see* Chilukuri et al). 1990;163:1201-2
Bonnar J (*see* Daly and Bonnar). 1990;163:430-7 (Symposium)
Booth RA Jr (*see* Sollenberger et al). 1990;163:1529-34
Boots L (*see* Brumfield et al). 1990;163:903-6
Bopp B (*see* Shoupe et al). 1990;163:1982-5
Borge D (*see* Gonsoulin et al). 1990;163:526-7
Borggaard B (*see* Refn et al). 1990;163:374-7 (Symposium)
Bosma PJ (*see* Kooistra et al). 1990;163:404-13 (Symposium)
Boucher M (*see* Marquette et al). 1990;163:1604-5
Bourgeois FJ, Duffer J. Outpatient obstetric management of women with type I diabetes. 1990;163:1065-73
Bowes WA Jr (*see* Katz et al). 1990;163:1142-3
—— (*see* Thorp and Bowes). 1990;163:1101-2 (Letter)
Bowie JD (*see* Hume et al). 1990;163:826-9
Boyle A (*see* Grifo et al). 1990;163:2013-9
Braak B (*see* Peterson and Braak). 1990;163:1247-8
Brace RA (*see* Piacquadio et al). 1990;163:1294-300
Brady K (*see* Christian et al). 1990;163:848-55
Branch DW, Dudley DJ, Mitchell MD, Creighton KA, Abbott TM, Hammond EH, Daynes RA. Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss. 1990;163:210-6
——, Scott JR. Pregnancy loss terminology. 1990;163:245-6 (Letter)
—— (*see* Dudley et al). 1990;163:1890-3
—— (*see* Rote et al). 1990;163:575-84
Brath PC (*see* Dickinson et al). 1990;163:1831-5
—— (*see* Roth et al). 1990;163:1828-30
Breckwoldt M, Wieacker P, Geisthövel F. Oral contraception in disease states. 1990;163:2213-6 (Symposium)
Brekus CA (*see* Romero et al). 1990;163:757-61
Bremmelgaard A (*see* Refn et al). 1990;163:374-7 (Symposium)
Bremner WJ (*see* Matsumoto et al). 1990;163:1743-51 (Symposium)
Brigandi A (*see* Lucifora et al). 1990;163:235-9
Briggs GG, Ambrose PJ, Nageotte MP. Cefotetan and the lack of associated bleeding. 1990;163:251-2 (Letter reply)
Brill K (*see* Düsterberg and Brill). 1990;163:1398-403 (Symposium)
—— (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Brody SA (*see* Goldzieher and Brody). 1990;163:2114-9 (Symposium)
Bronshstein M, Blumenfeld Z. Spontaneous resolution of a nuchal fetal cystic hygroma diagnosed early in the second trimester of pregnancy. 1990;163:268-9 (Letter reply)
Brooks MP (*see* Hogge et al). 1990;163:1061-5
Brophy MH. Zinc, preeclampsia, and γ-aminobutyric acid. 1990;163:242-3 (Letter)
Brown CEL, Christmas JT, Bawdon RE. Placental transfer of cefazolin and piperacillin in pregnancies remote from term complicated by Rh isoimmunization. 1990;163:938-43
——, Gant NF, Cox K, Spitz B, Rosenfeld CR, Magness RR. Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome. 1990;163:1853-61
Brown MA, Passaris G, Carlton MA. Pregnancy-induced hypertension and acute fatty liver of pregnancy: atypical presentations. 1990;163:1154-6
Brumfield CG, Cloud GA, Davis RO, Finley SC, Hauth JC, Boots L. The relationship between maternal serum and amniotic fluid α-fetoprotein in women undergoing early amniocentesis. 1990;163:903-6
Bruner JP (*see* Letterie et al). 1990;163:1100 (Letter)
Bruni V (*see* Abbate et al). 1990;163:424-9 (Symposium)
Bryson SCP (*see* Cavanagh et al). 1990;163:1007-15
Buchanan TA (*see* Kjos et al). 1990;163:93-8
Bukovsky I (*see* Langer et al). 1990;163:1139-41
—— (*see* Raziel et al). 1990;163:1182-5
Bull SB (*see* Morrow et al). 1990;163:1313-20
Bump RC (*see* Soper et al). 1990;163:1016-23
Burke AB, Hur D, Bolan JC, Corso P, Resano FG. Sinusoidal fetal heart rate pattern during cardiopulmonary bypass. 1990;163:17-8
Burkett G, Bandstra ES, Cohen J, Steele B, Palow D. Cocaine-related maternal death. 1990;163:40-1
Burns WJ (*see* Rizzo et al). 1990;163:1458-64
Burrows RF, Kelton JG. Low fetal risks in pregnancies associated with idiopathic thrombocytopenia purpura. 1990;163:1147-50
Butler WJ (*see* Odom et al). 1990;163:76-7
Byrd JR (*see* Tho et al). 1990;163:1968-75

C

Cabrera JA, Szekely Z, Ospitia JA. Suprapubic or vaginal procedure. 1990;163:2025 (Letter)
Cabrol D, Dubois C, Cronje H, Gonnet JM, Guillot M, Maria B, Moodley J, Oury JF, Thoulon JM, Treisser A, Ulmann D, Correl S, Ulmann A. Induction of labor with mifepristone (RU 486) in intrauterine fetal death. 1990; 163:540-2
Cadkin AV, McAlpin-Cadkin J. Ultrasonographic dating of very early pregnancy. 1990;163:1103 (Letter)
—— (*see* Ginsberg et al). 1990;163:1186-90
Caimi TM (*see* Baudo et al). 1990;163:1274-5
Calabro S (*see* Lucifora et al). 1990;163:235-9
Callahan R (*see* Romero et al). 1990;163:757-61
—— (*see* Romero et al). 1990;163:968-74
Cameron AD, Mathers AM, Wisdom S, Johnstone J, MacKenzie JRM, Walker JJ, Imrie SJ, Lowther GW, Connor JM. Second-trimester placental biopsy for rapid fetal karyotyping. 1990;163:931-4
Campaniello E (*see* Filicori et al). 1990;163:1737-42 (Symposium)
Campbell JM (*see* Taylor and Campbell). 1990;163:243 (Letter reply)
Campbell S (*see* Jauniaux and Campbell). 1990;163:1650-8
—— (*see* Jauniaux et al). 1990;163:1558-60
—— (*see* Rizzo et al). 1990;163:1231-8
Campbell WA (*see* Rodis et al). 1990;163:1168-71
Canick JA (*see* Haddow et al). 1990;163:1372 (Letter)
Cano LE (*see* Moise et al). 1990;163:776-84

Capeless EL (*see* Clapp and Capeless). 1990;163:1805-11
Cardin JP Jr, Ross MG, Ervin MG, Schaffer AV, Douglas FL, Simke JP. Fetal and maternal response to intravenous infusion of a thromboxane synthetase inhibitor. 1990;163:1345-9
Cardwell MS (*see* Snyder and Cardwell). 1990;163:244 (Letter reply)
Carey JC (*see* Klebanoff et al). 1990;163:1450-6
Carey KD (*see* Hankins et al). 1990;163:728-32
Caritis SN. Ritodrine infusion and cardiomyopathy. 1990;163:254-5 (Letter)
Carlan SJ, Papenhausen P, O'Brien WF, Pietrantoni M, Chez RA, Stephens P. Effect of maternal-fetal movement on concentration of cells obtained at genetic amniocentesis. 1990;163:490-3
Carlsen EC (*see* Sollenberger et al). 1990;163:1529-34
Carlson DE, Platt LD, Medearis AL, Horenstein J. Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus. 1990;163:1785-7
Carlton MA (*see* Brown et al). 1990;163:1154-6
Carmi R (*see* Meizner et al). 1990;163:128-9
Carpenter RJ Jr (*see* Gonsoulin et al). 1990;163:1172-3
—— (*see* Moise et al). 1990;163:776-84
—— (*see* Smith et al). 1990;163:567-9
Carr SR, Aronson MP, Coustan DR. Survival rates of monoamniotic twins do not decrease after 30 weeks' gestation. 1990;163:719-22
Carroccio G (*see* Lucifora et al). 1990;163:235-9
Carroll BA (*see* Hume et al). 1990;163:826-9
Cartter ML (*see* Rodis et al). 1990;163:1168-71
Cartwright W (*see* Mooney et al). 1990;163:30-6
Casaceli C (*see* Thompson et al). 1990;163:986-95
Caspi E (*see* Langer et al). 1990;163:1139-41
—— (*see* Raziel et al). 1990;163:1182-5
Cassidenti DL, Vijod AG, Vijod MA, Stanczyk FZ, Lobo RA. Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women. 1990;163:1953-60
Castillo RA (*see* Devoe et al). 1990;163:1040-8
Catanzarite V, Quirk JG. Second-trimester ultrasonography: determinants of visualization of fetal anatomic structures. 1990;163:1191-5
Cates W Jr (*see* Franks et al). 1990;163:1120-3
Cauley JA, Cummings SR, Black DM, Mascioli SR, Seeley DG. Prevalence and determinants of estrogen replacement therapy in elderly women. 1990;163:1438-44
Cavanagh D, Fiorica JV, Hoffman MS, Roberts WS, Bryson SCP, LaPolla JP, Barton DPJ. Invasive carcinoma of the vulva. 1990;163:1007-15
—— (*see* LaPolla et al). 1990;163:1055-60
Cederna JP, Davies BW, Farkas SA, Sonta JA, Sworniowski T. Necrotizing fasciitis of the total abdominal wall after sterilization by partial salpingectomy. 1990;163:138-9
Cefalo RC (*see* Thorp et al). 1990;163:655-6
Celnikier-Hochner D (*see* Ben-Chetrit et al). 1990;163:1161-3
Cerezo R (*see* Villar et al). 1990;163:151-7
Chaffin D (*see* Shah and Chaffin). 1990;163:1377 (Letter reply)
Challis JRG (*see* Cheung et al). 1990;163:1445-9
Chang V (*see* Xu et al). 1990;163:1493-7
Chao A. Graphic mnemonic for variable decelerations. 1990;163:1098 (Letter)
Chaouat G (*see* Szekeres-Bartho et al). 1990;163:1320-1
Chapman B (*see* Johnson et al). 1990;163:601-7
Chatterjee M (*see* Bardeguez et al). 1990;163:42-5
Chatwani A, Rech S, Mollick J. Clindamycin dosage scheduling for acute pelvic infection. 1990;163:240 (Letter)
Chaudhry A (*see* Naz et al). 1990;163:610-3
Chauhan SP. Does prophylactic amnioinfusion make a difference? 1990;163:1370 (Letter)
Chavkin W, Rosenfield A. A chill wind blows: Webster, obstetrics, and the health of women. 1990;163:450-2 (Clin. opinion)
Chazotte C, Cohen WR. Catastrophic complications of previous cesarean section. 1990;163:738-42
——, Girz B, Koenigsberg M, Cohen WR. Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis. 1990;163:1180-1
Check JH. One case of 45,X abortus with high CA 125 level. 1990;163:1717 (Letter)
Cheng H-M. Inhibitory cofactor of antiphospholipid antibody in normal pregnancy sera. 1990;163:701 (Letter)
Chervenak FA, McCullough LB. Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? 1990;163:1425-9 (Clin. opinion)
——, ——, Chervenak JL. Appropriate screening tests. 1990;163:688-9 (Letter reply)
——, ——, ——. Ethics and routine ultrasonography in pregnancy. 1990;163:257-8 (Letter reply)
Chervenak JL (*see* Chervenak et al). 1990;163:257-8 (Letter reply)
—— (*see* Chervenak et al). 1990;163:688-9 (Letter reply)
Chescheir NC (*see* Watson et al). 1990;163:1456-7
Chestnut DH. Epidural analgesia and cesarean section for dystocia. 1990;163:247-8 (Letter)
—— (*see* Pollack et al). 1990;163:787-94
Cheung CY (*see* Piacquadio et al). 1990;163:1294-300
Cheung PYC, Walton JC, Tai H-H, Riley SC, Challis JRG. Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta. 1990;163:1445-9
Chez RA (*see* Carlan et al). 1990;163:490-3
Chilukuri S, Bonet V, Cobb M. Antenatal spontaneous perforation of the extrahepatic biliary tree. 1990;163:1201-2
Chmielowiec S (*see* Dickinson et al). 1990;163:1831-5
Choi C-HM, Pritchard JR. Large cystic granulosa cell tumor: case report. 1990;163:74-6
Christian SS, Brady K, Read JA, Kopelman JN. Vaginal breech delivery: a five-year prospective evaluation of a protocol using computed tomographic pelvimetry. 1990;163:848-55
Christmas JT (*see* Brown et al). 1990;163:938-43
Chuganji Y (*see* Koike et al). 1990;163:696 (Letter)
Chuong CJ, Dawson EB, Smith ER. Vitamin E levels in premenstrual syndrome. 1990;163:1591-5
Cibils LA (*see* Lenox et al). 1990;163:1173-9
Clapp JF III. The course of labor after endurance exercise during pregnancy. 1990;163:1799-805
——, Capeless EL. Neonatal morphometrics after endurance exercise during pregnancy. 1990;163:1805-11
Clark KE (*see* Irion and Clark). 1990;163:222-9
Clark MR (*see* Van Voorhis et al). 1990;163:57-62
Clark RB. Definition of fetal asphyxia. 1990;163:1364 (Letter)
——, Quirk JG. What is birth asphyxia? 1990;163:1367-8 (Letter)
Clarke-Pearson DL (*see* Hunter et al). 1990;163:1164-7
—— (*see* Soper et al). 1990;163:1204-9
Clarkson TB (*see* Adams et al). 1990;163:1388-92 (Symposium)
Clewell WH (*see* Urig et al). 1990;163:1522-6

Clore JN (*see* Nestler et al). 1990;163:1243-6
Cloud GA (*see* Brumfield et al). 1990;163:903-6
Clouston WM (*see* Yap et al). 1990;163:477-8
Cobb M (*see* Chilukuri et al). 1990;163:1201-2
Cohen GR (*see* Thorp et al). 1990;163:607-9
Cohen J (*see* Burkett et al). 1990;163:40-1
—— (*see* Meizner et al). 1990;163:267-8 (Letter)
Cohen M (*see* Petrikovsky et al). 1990;163:253 (Letter reply)
Cohen WR (*see* Chazotte and Cohen). 1990;163:738-42
—— (*see* Chazotte et al). 1990;163:1180-1
Colditz GA (*see* Stampfer et al). 1990;163:285-91 (Symposium)
Collins JL (*see* Mutch et al). 1990;163:1920-4
Colvin EV (*see* Hankins et al). 1990;163:728-32
Combs CA, Robertson PA, Laros RK Jr. Risk factors for third-degree and fourth-degree perineal lacerations in forceps and vacuum deliveries. 1990;163:100-4
Condie RG (*see* George et al). 1990;163:1203-4
Conklin KA. Can anesthetic-related maternal mortality be reduced? 1990;163:253-4 (Letter)
Connor JM (*see* Cameron et al). 1990;163:931-4
Connor RE (*see* Smith et al). 1990;163:845-7
Constantine G (*see* George et al). 1990;163:1203-4
Cook EJ (*see* Macri et al). 1990;163:1248-53
—— (*see* Macri et al). 1990;163:1373-4 (Letter reply)
Cooper D (*see* Studd et al). 1990;163:1474-9
Copel JA, Schlafer D, Wentworth R, Belanger K, Kreitzer L, Hobbins JC, Nathanielsz PW. Does the umbilical artery systolic/diastolic ratio reflect flow or acidosis? 1990;163:751-6
Coppa G (*see* Esposito et al). 1990;163:572-4
Copper RL (*see* Owen et al). 1990;163:868-72
—— (*see* Owen et al). 1990;163:873-9
Correl S (*see* Cabrol et al). 1990;163:540-2
Corso P (*see* Burke et al). 1990;163:17-8
Corson SL. Sapphire probes in obstetric laser surgery. 1990;163:246-7 (Letter)
Cotton DB (*see* Smith et al). 1990;163:98-9
Coupland RE (*see* Johnson et al). 1990;163:601-7
Coustan DR (*see* Carr et al). 1990;163:719-22
—— (*see* Ojomo and Coustan). 1990;163:954-7
Covino JM, McCormack WM. Vulvar ulcer of unknown etiology in a human immunodeficiency virus–infected woman: response to treatment with zidovudine. 1990;163:116-8
Cox K (*see* Brown et al). 1990;163:1853-61
Cox SM, Sherman ML, Leveno KJ. Randomized investigation of magnesium sulfate for prevention of preterm birth. 1990;163:767-72
—— (*see* Cunningham et al). 1990;163:453-9
Coxon RJ (*see* Johnson et al). 1990;163:601-7
Crade M (*see* Harding et al). 1990;163:1566-8
—— (*see* Mills et al). 1990;163:1568-74
Cramer D (*see* Ewigman et al). 1990;163:256-7 (Letter)
Crandell SS (*see* Roth et al). 1990;163:1828-30
Crane JP (*see* Ewigman et al). 1990;163:256-7 (Letter)
Creighton KA (*see* Branch et al). 1990;163:210-6
—— (*see* Dudley et al). 1990;163:1890-3
Cronje H (*see* Cabrol et al). 1990;163:540-2
Crook D (*see* Godsland et al). 1990;163:348-53 (Symposium)
Cross RW. Breast diagnostics in clinical gynecology. 1990;163:686 (Letter)
Crosson JT (*see* Dayton et al). 1990;163:63-4
Crowley WF, Whitcomb RW. Gonadotropin-releasing hormone deficiency in men: diagnosis and treatment with exogenous gonadotropin-releasing hormone. 1990;163:1752-8 (Symposium)
Cruikshank SH (*see* Dayton et al). 1990;163:63-4
Cruz ML (*see* Stamler et al). 1990;163:1217-21
Cuckle HS (*see* Haddow et al). 1990;163:1372 (Letter)
Cullen JBH (*see* Sherer et al). 1990;163:1092 (Letter reply)
Cullen MT, Green J, Whetham J, Salafia C, Gabrielli S, Hobbins JC. Transvaginal ultrasonographic detection of congenital anomalies in the first trimester. 1990;163:466-76
—— (*see* Reece et al). 1990;163:162-9 (Curr. dev.)
Cummings SR (*see* Cauley et al). 1990;163:1438-44
Cummiskey KC, Dawood MY. Induction of labor with pulsatile oxytocin. 1990;163:1868-74
Cunningham FG, Cox SM, Harstad TW, Mason RA, Pritchard JA. Chronic renal disease and pregnancy outcome. 1990;163:453-9
—— (*see* Yeomans et al). 1990;163:958-64
Curet LB (*see* Izquierdo et al). 1990;163:584-7
Curiel P, Spinelli G, Petrella A, Gori A, De Maria R, Bonacina E, Gronda E. Postpartum coronary artery dissection followed by heart transplantation. 1990;163:538-9
Cuthrell WV, Rose JC, Meis PJ. The effect of adrenocorticotropic hormone infusion on subsequent pituitary response in the sheep fetus. 1990;163:170-4

D

d'Ablaing G (*see* Levin et al). 1990;163:543-4
Dallaire L (*see* Marquette et al). 1990;163:1604-5
Dalton KJ (*see* Mooney et al). 1990;163:30-6
D'Alton ME (*see* Garner et al). 1990;163:505-8
Daly L, Bonnar J. Comparative studies of 30 [mu]g ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets. 1990;163:430-7 (Symposium)
—— (*see* Hunter et al). 1990;163:1164-7
Damewood M (*see* Xu et al). 1990;163:1493-7
Damm P, Mølsted-Pedersen L. Ascertainment bias in diabetic outcome data. 1990;163:682-3 (Letter reply)
Damsbo P (*see* Beck-Nielsen et al). 1990;163:292-5 (Symposium)
Daniell JF (*see* Baggish and Daniell). 1990;163:247 (Letter reply)
Daru J, Mathur S. A comparison of sperm antibody assays. 1990;163:1622-9
David JL, Gaspard UJ, Gillain D, Raskinet R, Lepot MR. Hemostasis profile in women taking low-dose oral contraceptives. 1990;163:420-3 (Symposium)
Davidson S (*see* FitzSimmons and Davidson). 1990;163:682 (Letter)
Davies BW (*see* Cederna et al). 1990;163:138-9
Davis RO (*see* Brumfield et al). 1990;163:903-6
—— (*see* Owen et al). 1990;163:868-72
—— (*see* Owen et al). 1990;163:873-9
Dawood MY (*see* Cummiskey and Dawood). 1990;163:1868-74
—— (*see* Hecht et al). 1990;163:1986-91
Dawson EB (*see* Chuong et al). 1990;163:1591-5
Daynes RA (*see* Branch et al). 1990;163:210-6
Dayton VD, Anderson DS, Crosson JT, Cruikshank SH. A case of Rh isoimmunization: Should threatened first-trimester abortion be an indication for Rh immune globulin prophylaxis? 1990;163:63-4
Dean DD (*see* Rajabi et al). 1990;163:499-503
Dear C (*see* Devoe et al). 1990;163:1040-8
de Boer R (*see* Gevers Leuven et al). 1990;163:358-62 (Symposium)
—— (*see* Gevers Leuven et al). 1990;163:1410-3 (Symposium)
DeBruyn CS (*see* Pollack et al). 1990;163:787-94

deCataldo F (*see* Baudo et al). 1990;163:1274-5
de Catte L (*see* Foulon et al). 1990;163:1511-3
DeCherney AH (*see* Grifo et al). 1990;163:2013-9
Degani S. Fetal Doppler velocimetry in twins. 1990;163:1100-1 (Letter)
Delgado G (*see* Lewandowski et al). 1990;163:1333-7
Delke I (*see* Minkoff et al). 1990;163:521-6
De Maria R (*see* Curiel et al). 1990;163:538-9
Demers LM (*see* Wild et al). 1990;163:1998-2005
Dempsey LM (*see* Martin et al). 1990;163:1209-16
Dennis J, Johnson A, Mutch L, Yudkin P, Johnson P. Cerebral palsy and intrapartum fetal acidemia. 1990;163:267 (Letter reply)
de Onis M (*see* Villar et al). 1990;163:151-7
de Pasquale R (*see* Lucifora et al). 1990;163:235-9
De Ramundo B (*see* Petraglia et al). 1990;163:1307-12
Dersjant-Roorda MC (*see* Gevers Leuven et al). 1990;163:358-62 (Symposium)
—— (*see* Gevers Leuven et al). 1990;163:1410-3 (Symposium)
Desjacques P (*see* George et al). 1990;163:1203-4
DesJalais D (*see* Hoegsberg et al). 1990;163:1135-9
Desrochers M (*see* Marquette et al). 1990;163:1604-5
Devaskar S, McMenamy K, Holtzclaw L, Sadiq F. Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors. 1990;163:1350-6
Devoe LD, Gardner P, Dear C, Castillo RA. The diagnostic values of concurrent nonstress testing, amniotic fluid measurement, and Doppler velocimetry in screening a general high-risk population. 1990;163:1040-8
——, Murray C, Faircloth D, Ramos E. Vibroacoustic stimulation and fetal behavioral state in normal term human pregnancy. 1990;163:1156-61
Diamond MP (*see* Romero et al). 1990;163:968-74
Diaz F (*see* Kjos et al). 1990;163:898-903
Dickinson JE, Meyer BA, Brath PC, Chmielowiec S, Walsh SW, Parisi VM, Palmer SM. Placental thromboxane and prostacyclin production in an ovine diabetic model. 1990;163:1831-5
Dickson KA (*see* Harding et al). 1990;163:1904-13
Dietl J, Lippert TH. Critical comments on the treatment of tubal pregnancy with prostaglandins. 1990;163:707 (Letter)
Digre KB, Varner MW, Schiffman JS. Neuroophthalmologic effects of intravenous magnesium sulfate. 1990;163:1848-52
Dilks SA (*see* Hogge et al). 1990;163:1061-5
Dillon HC (*see* Silver et al). 1990;163:19-25
Dimitry ES, Oskarsson T, Margara R, Winston RML. Heterotopic pregnancy associated with assisted reproductive technology. 1990;163:244-5 (Letter)
DiSciullo AJ (*see* Korn et al). 1990;163:596-8
Divers MJ. Ketoconazole treatment of Cushing's syndrome in pregnancy. 1990;163:1101 (Letter)
Divon MY. Fetal Doppler velocimetry in twins. 1990;163:1101 (Letter reply)
Dodd GD, McLelland R. Breast disease programs and mammography training. 1990;163:689-90 (Letter)
Dodds WG (*see* Goldberg et al). 1990;163:65-8
Dodson MG (*see* Juneja and Dodson). 1990;163:216-21
—— (*see* Roudebush et al). 1990;163:1670-3
Doellgast GJ (*see* Kutteh et al). 1990;163:1301-6
Dombroski RA, Woodard DS, Harper MJK, Gibbs RS. A rabbit model for bacteria-induced preterm pregnancy loss. 1990;163:1938-43
Dommisse J. Phenytoin and magnesium sulfate in eclampsia. 1990;163:1089-90 (Letter)
Dooley SL (*see* Keller et al). 1990;163:893-7
—— (*see* Utter et al). 1990;163:882-6
—— (*see* Utter et al). 1990;163:1781-4
Dorche C (*see* George et al). 1990;163:1203-4
Dorchester W (*see* Asrat et al). 1990;163:887-9
—— (*see* Mills et al). 1990;163:1568-74
Dostal-Johnson D (*see* Rote et al). 1990;163:575-84
Douglas FL (*see* Cardin et al). 1990;163:1345-9
Douyan S (*see* Kjos et al). 1990;163:1822-7
Downing JW, Knape KG. Labor epidural analgesia and dystocia-related cesarean section. 1990;163:1096-7 (Letter)
Drake RF (*see* Yap et al). 1990;163:477-8
Droste S, FitzSimmons J, Pascoe-Mason J, Shepard TH. Growth of linear parameters in trisomy 18 fetuses. 1990;163:158-61
Drugan A (*see* Johnson et al). 1990;163:1505-10
Druzin ML (*see* August et al). 1990;163:1612-21
Dubois C (*see* Cabrol et al). 1990;163:540-2
Dudley AG (*see* Wiskind et al). 1990;163:1674-8
Dudley DJ, Mitchell MD, Creighton K, Branch DW. Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells. 1990;163:1890-3
—— (*see* Branch et al). 1990;163:210-6
Dudley DK (*see* Garner et al). 1990;163:505-8
Duffer J (*see* Bourgeois and Duffer). 1990;163:1065-73
Dungan JS (*see* Hogge et al). 1990;163:1061-5
Dunn LK. Fetal heart rate monitoring during cesarean section. 1990;163:253 (Letter)
Dupont P, Irion O, Béguin F. Pregnancy in a patient with treated Wilson's disease: a case report. 1990;163:1527-8
Durukan T, Urman B, Yarali H, Arikan Ü, Beykal Ö. An abdominal pregnancy 10 years after treatment for pelvic tuberculosis. 1990;163:594-5
Düsterberg B, Brill K. Clinical acceptability of monophasic gestodene. 1990;163:1398-403 (Symposium)
Dyer DC (*see* Isla and Dyer). 1990;163:1337-44
Dyson DC (*see* Ferguson et al). 1990;163:105-11

E

Easterling TR, Benedetti TJ. Measurement of cardiac output by impedance technique. 1990;163:1104-6 (Letter)
Edersheim TG (*see* August et al). 1990;163:1612-21
Egarter Ch. The complex nature of egg transport through the oviduct. 1990;163:687 (Letter)
——, Husslein P. Critical comments on the treatment of tubal pregnancy with prostaglandins. 1990;163:707-8 (Letter reply)
——, ——, Rayburn WF. Uterine hyperstimulation after low-dose prostaglandin E_2 therapy: tocolytic treatment in 181 cases. 1990;163:794-6
Egerman RS (*see* Johnson et al). 1990;163:1024-32
Eierman D (*see* Kauma et al). 1990;163:1430-7
Eigenbrodt EH (*see* Yeomans et al). 1990;163:958-64
Elbourne D, Grant A. Study results vary in count-to-10 method of fetal movement screening. 1990;163:264 (Letter)
Elder MG (*see* Bennett et al). 1990;163:241-2 (Letter)
Elias AN, Varizi ND. Plasma atrial arginine vasopressin levels vary little between preeclamptic and control patients. 1990;163:245 (Letter)
Elias S (*see* Shulman et al). 1990;163:1606-9
Ellington JR Jr (*see* Orr et al). 1990;163:1485-9
Elliot DL. Use of the T-ACE questions to detect risk-drinking. 1990;163:684 (Letter)
Elliott JP (*see* Mills et al). 1990;163:1568-74

—— (*see* Urig et al). 1990;163:1522-6
Emert MP (*see* Mader et al). 1990;163:1578-9
Erkkola RU (*see* Mäkinen et al). 1990;163:266 (Letter reply)
Ernest JM, Givner LB, Pool R. Intrapartum hepatitis B screening in a low-risk population. 1990;163:978-80
Ervin MG (*see* Cardin et al). 1990;163:1345-9
Escamilla JO. Use of midforceps applauded. 1990;163:2027 (Letter)
Eschenbach DA (*see* Harger et al). 1990;163:130-7
Esposito RA, Grossi EA, Coppa G, Giangola G, Ferri DP, Angelides EM, Andriakos P. Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy. 1990;163:572-4
Estañ L (*see* Martinez-Mir et al). 1990;163:189-95
Evans AT (*see* Gillogley et al). 1990;163:1535-42
Evans MI (*see* Johnson et al). 1990;163:1505-10
Evans WS (*see* Sollenberger et al). 1990;163:1529-34
Ewigman B, LeFevre M, Bain RP, Crane JP, Frigoletto F, Cramer D, McNellis D. Ethics and routine ultrasonography in pregnancy. 1990;163:256-7 (Letter)

F

Faircloth D (*see* Devoe et al). 1990;163:1156-61
Fairlie FM (*see* Moretti et al). 1990;163:1844-8
—— (*see* Sibai et al). 1990;163:733-8
Falciglia HS. Amnioinfusion and prevention of meconium aspiration syndrome. 1990;163:258-9 (Letter)
——. Is meconium aspiration an intrauterine event? 1990;163:250 (Letter)
Faleschini E (*see* Rovati et al). 1990;163:1490-2
Fallenstein F (*see* Spätling and Fallenstein). 1990;163:240-1 (Letter)
Farkas SA (*see* Cederna et al). 1990;163:138-9
Faro S (*see* Martens and Faro). 1990;163:251 (Letter)
Farrell M (*see* Lewandowski et al). 1990;163:1333-7
Favretti L (*see* Puppa et al). 1990;163:701-2 (Letter)
Federici MG (*see* Rubin et al). 1990;163:69-73
Feichtinger W. Prolongation of pregnancy and survival of second twin. 1990;163:270-1 (Letter reply)
Feldman J (*see* Hoegsberg et al). 1990;163:1135-9
—— (*see* Minkoff et al). 1990;163:521-6
Feldman N, Skoll A, Sibai B. The incidence of significant fetomaternal hemorrhage in patients undergoing cesarean section. 1990;163:855-8
Ferenczy A, Bergeron C, Richart RM. Carbon dioxide laser energy disperses human papillomavirus deoxyribonucleic acid onto treatment fields. 1990;163:1271-4
Ferguson JE (*see* Hogge et al). 1990;163:1061-5
Ferguson JE II, Dyson DC, Schutz T, Stevenson DK. A comparison of tocolysis with nifedipine or ritodrine: analysis of efficacy and maternal, fetal, and neonatal outcome. 1990;163:105-11
——, Vick DJ, Hogge JS, Hogge WA. Transcervical chorionic villus sampling and amniocentesis: a comparison of reliability, culture findings, and fetal outcome. 1990;163:926-31
Ferguson JF II (*see* Hogge et al). 1990;163:698 (Letter reply)
Ferguson R, Myers SA. The effect of race on the relationship between fetal death and altered fetal growth. 1990;163:1222-30
Ferrari P (*see* Filicori et al). 1990;163:1737-42 (Symposium)
Ferri DP (*see* Esposito et al). 1990;163:572-4
Fertel RH (*see* Kniss et al). 1990;163:1883-90
Fields M (*see* Fuchs et al). 1990;163:1961-7
Figueroa JP, Barbera M, Honnebier OM, Jenkins S, Nathanielsz PW. Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle. 1990;163:648-54
Filicori M, Flamigni C, Campaniello E, Meriggiola MC, Michelacci L, Valdiserri A, Ferrari P. Polycystic ovary syndrome: abnormalities and management with pulsatile gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs. 1990;163:1737-42 (Symposium)
Fink DJ (*see* Matera et al). 1990;163:797-801
Finley SC (*see* Brumfield et al). 1990;163:903-6
Finstad CL (*see* Rubin et al). 1990;163:69-73
Fiorica JV (*see* Cavanagh et al). 1990;163:1007-15
—— (*see* LaPolla et al). 1990;163:1055-60
Fischer E (*see* Grifo et al). 1990;163:2013-9
Fisher DJ (*see* Moise et al). 1990;163:776-84
Fisk NM (*see* Nicolini et al). 1990;163:1104 (Letter reply)
—— (*see* Nicolini et al). 1990;163:1144-6
Fitzpatrick RJ (*see* Owiny and Fitzpatrick). 1990;163:657-60
FitzSimmons J, Davidson S. Ascertainment bias in diabetic outcome data. 1990;163:682 (Letter)
—— (*see* Droste et al). 1990;163:158-61
Flamigni C (*see* Filicori et al). 1990;163:1737-42 (Symposium)
Flamm BL. Vaginal birth after cesarean section: an appraisal of the reappraisal. 1990;163:691 (Letter)
Fliegner JR, Garland SM. Perinatal mortality in Victoria, Australia: role of group B streptococcus. 1990;163:1609-11
Flor S (*see* McGuire et al). 1990;163:2127-31 (Symposium)
Flouret G (*see* Wilson et al). 1990;163:195-202
—— (*see* Wilson et al). 1990;163:1875-82
Flu PK (*see* Akkersdijk et al). 1990;163:591-3
Fogelman I (*see* Studd et al). 1990;163:1474-9
Fong K, Ohlsson A, Zalev A. Fetal thoracic circumference: a prospective cross-sectional study with real-time ultrasound (1988;158:1154-60). 1990;163:1363 (Correction)
Foreman F (*see* Benzaquen et al). 1990;163:484-90
Forman A (*see* Skajaa et al). 1990;163:1323-33
Fossum G (*see* Shoupe et al). 1990;163:1982-5
Fotherby K. Interactions with oral contraceptives. 1990;163:2153-9 (Symposium)
——. Pharmacokinetics of gestagens: some problems. 1990;163:323-8 (Symposium)
Foulon W, Naessens A, de Catte L, Amy J-J. Detection of congenital toxoplasmosis by chorionic villus sampling and early amniocentesis. 1990;163:1511-3
Fox HE (*see* Berkowitz et al). 1990;163:975-7
—— (*see* Matera et al). 1990;163:797-801
Franco-Buff A (*see* McGregor et al). 1990;163:1465-73
Franks AL, Beral V, Cates W Jr, Hogue CJR. Contraception and ectopic pregnancy risk. 1990;163:1120-3
—— (*see* Saftlas et al). 1990;163:460-5
Fredstrom GR (*see* Smith et al). 1990;163:845-7
Freedman WL (*see* Stark et al). 1990;163:818-23
Freinkel N (*see* Keller et al). 1990;163:893-7
—— (*see* Martin et al). 1990;163:1209-16
—— (*see* Rizzo et al). 1990;163:1458-64
French JI (*see* McGregor et al). 1990;163:1465-73
—— (*see* McGregor et al). 1990;163:1580-91
Frieden FJ, Ordorica SA, Hoskins IA, Young BK. The Shirodkar operation: a reappraisal. 1990;163:830-3
—— (*see* Hoskins et al). 1990;163:1944-7
Friedman AJ, Lobel SM, Rein MS, Barbieri RL. Efficacy and safety considerations in women with uterine leiomyomas treated with gonadotropin-releasing hormone agonists: the

estrogen threshold hypothesis. 1990;163:1114-9 (Clin. opinion)
Friedman CI (*see* Goldberg et al). 1990;163:65-8
Friedman JM (*see* Gilchrist et al). 1990;163:692-3 (Letter)
Friedman RL (*see* Kjos et al). 1990;163:1822-7
Friedman WW (*see* Towers et al). 1990;163:965-7
Frigoletto F (*see* Ewigman et al). 1990;163:256-7 (Letter)
Frohlich EP. Combination therapy has potential complications. 1990;163:244 (Letter)
Fuchs A-R, Behrens O, Helmer H, Vangsted A, Ivanisevic M, Grifo J, Fields M, Barros C. Oxytocin and vasopressin binding sites in human and bovine ovaries. 1990;163:1961-7
Fujimoto S (*see* Koga et al). 1990;163:1543-5

G

Gabbe SG (*see* Kniss et al). 1990;163:1883-90
Gabrielli S (*see* Cullen et al). 1990;163:466-76
—— (*see* Reece et al). 1990;163:162-9 (Curr. dev.)
Gagnon R, Hunse C, Vijan S. The effect of maternal hyperoxia on behavioral activity in growth-retarded human fetuses. 1990;163:1894-9
—— (*see* Benzaquen et al). 1990;163:484-90
Gail MH (*see* Minkoff et al). 1990;163:1598-604
Gale S (*see* Moore et al). 1990;163:907-12
Gall M (*see* Hume et al). 1990;163:826-9
Gambone JC, Reiter RC, Lench JB, Moore JG. The impact of a quality assurance process on the frequency and confirmation rate of hysterectomy. 1990;163:545-50
Gandell DL (*see* Sites et al). 1990;163:1630-1
Gant NF (*see* Brown et al). 1990;163:1853-61
Garat E (*see* Simon et al). 1990;163:382-7 (Symposium)
Gardner P (*see* Devoe et al). 1990;163:1040-8
Garite TJ (*see* Asrat et al). 1990;163:887-9
—— (*see* Lewis et al). 1990;163:773-5
—— (*see* Towers et al). 1990;163:965-7
Garland SM (*see* Fliegner and Garland). 1990;163:1609-11
Garner PR, D'Alton ME, Dudley DK, Huard P, Hardie M. Preeclampsia in diabetic pregnancies. 1990;163:505-8
Garnett T (*see* Studd et al). 1990;163:1474-9
Garnier P (*see* Simon et al). 1990;163:382-7 (Symposium)
Garuti GC (*see* Petraglia et al). 1990;163:1307-12
Gary GW Jr (*see* Rodis et al). 1990;163:1168-71
Gaspard UJ, Lefebvre PJ. Clinical aspects of the relationship between oral contraceptives, abnormalities in carbohydrate metabolism, and the development of cardiovascular disease. 1990;163:334-43 (Symposium)
—— (*see* David et al). 1990;163:420-3 (Symposium)
—— (*see* Jandrain et al). 1990;163:378-81 (Symposium)
Gast MJ (*see* Long et al). 1990;163:1976-81
Gates HS Jr. Definition of primparas. 1990;163:677 (Letter)
Geisthövel F (*see* Breckwoldt et al). 1990;163:2213-6 (Symposium)
Genazzani AR (*see* Petraglia et al). 1990;163:1307-12
Gennser G (*see* Stale et al). 1990;163:26-9
George R, Gray F, Green A, Basu SN, Constantine G, Condie RG, Dorche C, Vianey-Liaud C, Desjacques P. Antenatal diagnosis of molybdenum cofactor deficiency. 1990;163:1203-4
Gevers Leuven JA, Dersjant-Roorda MC, Helmerhorst FM, de Boer R, Neymeyer-Leloux A, Havekes L. Estrogenic effect of gestodene-or desogestrel-containing oral contraceptives on lipoprotein metabolism. 1990;163:358-62 (Symposium)
——, ——, ——, ——, ——, ——. Effects of oral contraceptives on lipid metabolism. 1990;163:1410-3 (Symposium)
Giangola G (*see* Esposito et al). 1990;163:572-4
Giard RWM (*see* Akkersdijk et al). 1990;163:591-3
Gibb D (*see* Jauniaux et al). 1990;163:1558-60
Gibbs RS (*see* Dombroski et al). 1990;163:1938-43
—— (*see* Harger et al). 1990;163:130-7
—— (*see* Silver et al). 1990;163:19-25
—— (*see* Stark et al). 1990;163:818-23
Gifford RW Jr (*see* Lenfant et al). 1990;163:2113 (Symposium)
Gilbert WM, Lafferty CM, Benirschke K, Resnik R. Lack of specific placental abnormality associated with cocaine use. 1990;163:998-9
Gilchrist DM, Friedman JM, Wilson RD. Maternal serum α-fetoprotein in pregnancy. 1990;163:692-3 (Letter)
Giles W, Trudinger B, Wilcox G. Discrepancies in hemoglobin levels. 1990;163:1713 (Letter)
Gill TJ III (*see* Ho et al). 1990;163:178-88
Gillain D (*see* David et al). 1990;163:420-3 (Symposium)
Gillogley KM, Evans AT, Hansen RL, Samuels SJ, Batra KK. The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening. 1990;163:1535-42
Gilstrap LC III (*see* Harstad et al). 1990;163:912-6
—— (*see* Roe et al). 1990;163:715-8
Gimbel GL. Can shoulder dystocia be predicted? 1990;163:680 (Letter)
——. Colposcopy in diethylstilbestrol-exposed offspring. 1990;163:703 (Letter)
Ginsberg N, Cadkin A, Pergament E, Verlinsky Y. Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21. 1990;163:1186-90
Girz B (*see* Chazotte et al). 1990;163:1180-1
Givner LB (*see* Ernest et al). 1990;163:978-80
Glassman MB (*see* Lipper et al). 1990;163:146-50
Gleicher N. Breast diagnostics in clinical gynecology. 1990;163:687 (Letter reply)
——. Breast disease programs and mammography training. 1990;163:690-1 (Letter reply)
Glezerman M, Piura B, Insler V. Trend of cervical cancer in Jewish women. 1990;163:704 (Letter reply)
Gocke SE (*see* Asrat et al). 1990;163:887-9
Godsland IF, Crook D, Wynn V. Low-dose oral contraceptives and carbohydrate metabolism. 1990;163:348-53 (Symposium)
Goedett JJ (*see* Minkoff et al). 1990;163:1598-604
Golan A (*see* Langer et al). 1990;163:1139-41
—— (*see* Raziel et al). 1990;163:1182-5
Golbus J (*see* Silver et al). 1990;163:1914-9
Golbus MS (*see* Pinckert et al). 1990;163:684 (Letter reply)
Goldberg JM, Haering PL, Friedman CI, Dodds WG, Kim MH. Antisperm antibodies in women undergoing intrauterine insemination. 1990;163:65-8
Goldbloom DS (*see* Stewart et al). 1990;163:1196-9
Goldenberg RL (*see* Owen et al). 1990;163:868-72
—— (*see* Owen et al). 1990;163:873-9
Goldstein I, Wise GJ, Tancer ML. A vesicovaginal fistula and intravesical foreign body: a rare case of the neglected pessary. 1990;163:589-91
——, Zimmer EZ, Merzbach D, Peretz BA, Paldi E. Intraamniotic infection in the very early phase of the second trimester. 1990;163:1261-3
Goldstein SR. Early pregnancy failure—appropriate terminology. 1990;163:1093 (Letter)
——. Vaginal probe ultrasonography and ectopic pregnancy. 1990;163:1099 (Letter)
——, Nachtigall M, Snyder JR, Nachtigall L. Endometrial as-

sessment by vaginal ultrasonography before endometrial sampling in patients with postmenopausal bleeding. 1990;163:119-23
Goldzieher JW. Selected aspects of the pharmacokinetics and metabolism of ethinyl estrogens and their clinical implications. 1990;163:318-22 (Symposium)
——, Brody SA. Pharmacokinetics of ethinyl estradiol and mestranol. 1990;163:2114-9 (Symposium)
Gonnet JM (*see* Cabrol et al). 1990;163:540-2
Gonsoulin W, Borge D, Moise KJ Jr. Rupture of unscarred uterus in primigravid woman in association with cocaine abuse. 1990;163:526-7
——, Mason B, Carpenter RJ Jr. Colon cancer in pregnancy with elevated maternal serum α-fetoprotein level at presentation. 1990;163:1172-3
—— (*see* Smith et al). 1990;163:567-9
Gonzalez P (*see* Nicolini et al). 1990;163:1144-6
Goodlin RC. Cerebral palsy and intrapartum fetal acidemia. 1990;163:266-7 (Letter)
——. Digoxin-like immunoreactive factor and respiratory distress syndrome. 1990;163:1091 (Letter)
——. HELLP does not always mean immediate HELP! 1990;163:1089 (Letter)
——. Intracellular sodium levels in pregnant women with hypertension. 1990;163:261-2 (Letter)
——. Magnesium sulfate is not an ideal anticonvulsant. 1990;163:1714 (Letter)
——. Prolongation of pregnancy and survival of second twin. 1990;163:270 (Letter)
Gori A (*see* Curiel et al). 1990;163:538-9
Gorstein F (*see* Osteen et al). 1990;163:479-84
Gouyon JB, Petion AM. Toxicity of vancomycin given during pregnancy. 1990;163:1375 (Letter)
Gram J, Munkvad S, Jespersen J. Enhanced generation and resolution of fibrin in women above the age of 30 years using oral contraceptives low in estrogen. 1990;163:438-42 (Symposium)
Grand BE (*see* Riveros et al). 1990;163:704-5 (Letter)
Grant A (*see* Elbourne and Grant). 1990;163:264 (Letter)
Gray BM (*see* Silver et al). 1990;163:19-25
Gray F (*see* George et al). 1990;163:1203-4
Greelish JP (*see* Hatjis et al). 1990;163:45-50
Green A (*see* George et al). 1990;163:1203-4
Green J (*see* Cullen et al). 1990;163:466-76
Green JR, Pawson IG, Schumacher LB, Perry J, Kretchmer N. Glucose tolerance in pregnancy: ethnic variation and influence of body habitus. 1990;163:86-92
Greenberg F (*see* Smith et al). 1990;163:567-9
Greenspoon JS. Ultrasonographic guidance for central venous access. 1990;163:252 (Letter)
—— (*see* Kjos et al). 1990;163:93-8
Greisman B (*see* Wilansky and Greisman). 1990;163:263 (Letter reply)
—— (*see* Wilansky and Greisman). 1990;163:697 (Letter reply)
Grifo J (*see* Fuchs et al). 1990;163:1961-7
Grifo JA, Boyle A, Fischer E, Lavy G, DeCherney AH, Ward DC, Sanyal MK. Preembryo biopsy and analysis of blastomeres by in situ hybridization. 1990;163:2013-9
Grimes DA (*see* Levin et al). 1990;163:543-4
Gronda E (*see* Curiel et al). 1990;163:538-9
Grose C, Weiner CP. Prenatal diagnosis of congenital cytomegalovirus infection: two decades later. 1990;163:447-50 (Clin. opinion)
—— (*see* Weiner and Grose). 1990;163:1253-5
Gross KM (*see* Matsumoto et al). 1990;163:1743-51 (Symposium)
Gross W (*see* Kuhl et al). 1990;163:363-9 (Symposium)
Grossi EA (*see* Esposito et al). 1990;163:572-4
Grover JL (*see* La Puma et al). 1990;163:680 (Letter reply)
Guengerich FP. Inhibition of oral contraceptive steroid-metabolizing enzymes by steroids and drugs. 1990;163:2159-63 (Symposium)
Guillebaud J. Oral contraceptives in risk groups: Exclusion or monitoring? 1990;163:443-6 (Symposium)
Guillot M (*see* Cabrol et al). 1990;163:540-2
Guy GP, Peisner DB, Timor-Tritsch IE. Ultrasonographic evaluation of uteroplacental blood flow patterns of abnormally located and adherent placentas. 1990;163:723-7

H

Haas ST. Caution in use of new test for ectopic pregnancy. 1990;163:694 (Letter)
Haddow JE, Palomaki GE, Knight GJ, Canick JA, Wald NJ, Cuckle HS. Maternal serum unconjugated estriol levels are lower in the presence of fetal Down syndrome. 1990;163:1372 (Letter)
—— (*see* Johnson et al). 1990;163:9-11
Hadi HA, Albazzaz SJ. Effects of ritodrine tocolysis on cardiac isozymes and electrocardiography. 1990;163:700-1 (Letter reply)
Haering PL (*see* Goldberg et al). 1990;163:65-8
Hagay Z (*see* Romero et al). 1990;163:823-5
Hahlin M (*see* Lindblom et al). 1990;163:260-1 (Letter reply)
—— (*see* Lindblom et al). 1990;163:694-5 (Letter reply)
Hahn D (*see* McGuire et al). 1990;163:2127-31 (Symposium)
Hakes TB (*see* Rubin et al). 1990;163:69-73
Halim MA (*see* Al Kasab et al). 1990;163:37-40
Hambley H (*see* Blesing and Hambley). 1990;163:697 (Letter)
Hammerstedt RH (*see* Vasquez et al). 1990;163:2006-12
Hammerstein J. Prodrugs: Advantage or disadvantage? 1990;163:2198-203 (Symposium)
Hammond EH (*see* Branch et al). 1990;163:210-6
Hanaoka S (*see* Romero et al). 1990;163:968-74
Handberg [oc]A (*see* Beck-Nielsen et al). 1990;163:292-5 (Symposium)
Hanker JP. Gastrointestinal disease and oral contraception. 1990;163:2204-7 (Symposium)
Hankins GDV, Lowery CL Jr, Scott RT, Morrow WR, Carey KD, Leland MM, Colvin EV. Transplacental transfer of zidovudine in the near-term pregnant baboon. 1990;163:728-32
—— (*see* Strickland et al). 1990;163:4-8 (Clin. opinion)
Hansen RL (*see* Gillogley et al). 1990;163:1535-42
Harbert GM Jr (*see* Hogge et al). 1990;163:698 (Letter reply)
Hardardottir H, Parmley TH II, Quirk JG Jr, Sanders MM, Miller FC, O'Brien TJ. Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm. 1990;163:1925-31
Hardie M (*see* Garner et al). 1990;163:505-8
Harding JA, Lewis D, Major C, Crade M, Patel J, Nageotte M. Color flow Doppler—a useful instrument in the diagnosis of vasa previa. 1990;163:1566-8
Harding R, Hooper SB, Dickson KA. A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios. 1990;163:1904-13
Harger JH, Hsing AW, Toumala RE, Gibbs RS, Mead PB, Eschenbach DA, Knox GE, Polk BF. Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study. 1990;163:130-7

Hargrove JT (*see* Osteen et al). 1990;163:479-84
Harkavy KL. Mortality rates ignore population segment. 1990;163:269 (Letter)
Harper MJK (*see* Dombroski et al). 1990;163:1938-43
Harris EN (*see* Reece et al). 1990;163:162-9 (Curr. dev.)
Harstad TW, Little BB, Bawdon RE, Knoll K, Roe D, Gilstrap LC III. Embryofetal effects of pentamidine isethionate administered to pregnant Sprague-Dawley rats. 1990;163:912-6
—— (*see* Cunningham et al). 1990;163:453-9
Hatcher R (*see* Rizzo et al). 1990;163:1458-64
Hatjis CG, Kofinas AD, Greelish JP, Swain M, Rose JC. Interrelationship between atrial natriuretic factor concentrations and acute volume expansion in pregnant and nonpregnant women. 1990;163:45-50
Hattori T (*see* Koga et al). 1990;163:1543-5
Hauth JC (*see* Brumfield et al). 1990;163:903-6
—— (*see* Owen et al). 1990;163:868-72
Havekes LM (*see* Gevers Leuven et al). 1990;163:358-62 (Symposium)
—— (*see* Gevers Leuven et al). 1990;163:1410-3 (Symposium)
Hawkins RL (*see* Bamford et al). 1990;163:661-8
Hayashi RH (*see* Andersen et al). 1990;163:859-67
Hazzard W (*see* Wild et al). 1990;163:1998-2005
Hecht BR, Bardawil WA, Khan-Dawood FS, Dawood MY. Luteal insufficiency: correlation between endometrial dating and integrated progesterone output in clomiphene citrate–induced cycles. 1990;163:1986-91
Heidt F (*see* Kuhl et al). 1990;163:363-9 (Symposium)
Heilbron DC (*see* Taylor et al). 1990;163:1839-44
Heithecker R (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Helmer H (*see* Fuchs et al). 1990;163:1961-7
Helmerhorst FM (*see* Gevers Leuven et al). 1990;163:358-62 (Symposium)
—— (*see* Gevers Leuven et al). 1990;163:1410-3 (Symposium)
Helmerhorst TJM. Colposcopy in diethylstilbestrol-exposed offspring. 1990;163:703 (Letter reply)
Helmkamp BF, Krebs H-B. The Maylard incision in gynecologic cancer. 1990;163:1554-7
Helms M (*see* Hunter et al). 1990;163:1164-7
Henderson C (*see* Minkoff et al). 1990;163:1598-604
Hendricks SK, Katz M. Effects of ritodrine tocolysis on cardiac isozymes and electrocardiography. 1990;163:699-700 (Letter)
Hendrix PV (*see* Lazebnik et al). 1990;163:78-80
Hennekens CH (*see* Stampfer et al). 1990;163:285-91 (Symposium)
Henriksen JE (*see* Beck-Nielsen et al). 1990;163:292-5 (Symposium)
Hertzberg BS (*see* Hume et al). 1990;163:826-9
Hetzler G (*see* Strong et al). 1990;163:1370-1 (Letter reply)
Hickok DE (*see* Mahony et al). 1990;163:1513-22
Hill GA (*see* Osteen et al). 1990;163:479-84
Hill GB (*see* Livengood et al). 1990;163:515-20
Hill JA (*see* Van Voorhis et al). 1990;163:57-62
Hillier S (*see* McGregor et al). 1990;163:1465-73
—— (*see* McGregor et al). 1990;163:1580-91
Hinman DJ (*see* Szeto and Hinman). 1990;163:202-9
Hirsch JH (*see* Mahony et al). 1990;163:1513-22
Hirvonen E, Idänpään-Heikkilä J. Cardiovascular death among women under 40 years of age using low-estrogen oral contraceptives and intrauterine devices in Finland from 1975 to 1984. 1990;163:281-4 (Symposium)
Ho H-N, Gill TJ III, Nsieh R-P, Hsieh H-J, Lee T-Y. Sharing of human leukocyte antigens in primary and secondary recurrent spontaneous abortions. 1990;163:178-88
Hobart ED (*see* Silver et al). 1990;163:917-22
Hobbins JC (*see* Copel et al). 1990;163:751-6
—— (*see* Cullen et al). 1990;163:466-76
—— (*see* Reece et al). 1990;163:162-9 (Curr. dev.)
—— (*see* Romero et al). 1990;163:757-61
—— (*see* Romero et al). 1990;163:823-5
—— (*see* Romero et al). 1990;163:968-74
Hoegsberg B, Abulafia O, Sedlis A, Feldman J, DesJalais D, Landesman S, Minkoff H. Sexually transmitted diseases and human immunodeficiency virus infection among women with pelvic inflammatory disease. 1990;163:1135-9
Hoffman DR (*see* McCoshen et al). 1990;163:1632-40
Hoffman MS (*see* Cavanagh et al). 1990;163:1007-15
—— (*see* LaPolla et al). 1990;163:1055-60
Hogge JS (*see* Ferguson et al). 1990;163:926-31
Hogge WA, Dungan JS, Brooks MP, Dilks SA, Abbitt PL, Thiagarajah S, Ferguson JE. Diagnosis and management of prenatally detected myelomeningocele: a preliminary report. 1990;163:1061-5
——, Thiagarajah S, Ferguson JF II, Schnatterly PT, Harbert GM Jr. Should amniocenteses always accompany unexplained high maternal serum α-fetoprotein levels? Who decides: public agency, patient, or private physician? 1990;163:698 (Letter reply)
—— (*see* Ferguson et al). 1990;163:926-31
Hogue CJR (*see* Franks et al). 1990;163:1120-3
Holloway RW (*see* Lewandowski et al). 1990;163:1333-7
Holman S (*see* Minkoff et al). 1990;163:1598-604
Holtzclaw L (*see* Devaskar et al). 1990;163:1350-6
Honnebier OM (*see* Figueroa et al). 1990;163:648-54
Hooper SB (*see* Harding et al). 1990;163:1904-13
Hoppe G. The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction. 1990;163:388-91 (Symposium)
Hoppichler F (*see* Loewit et al). 1990;163:708-10 (Letter)
Horenstein J (*see* Carlson et al). 1990;163:1785-7
Horger EO III (*see* Arnett et al). 1990;163:1074-80
Horimoto N, Koyanagi T, Satah S, Yoshizato T, Nakano H. Fetal eye movement assessed with real-time ultrasonography: Are there rapid and slow eye movements? 1990;163:1480-4
Horowitz M (*see* Nordin et al). 1990;163:140-5
Hoskins IA, Katz J, Frieden FJ, Ordorica SA, Young BK. In vitro inhibition of esterase activity in amniotic fluid: comparison with bacterial cultures. 1990;163:1944-7
—— (*see* Frieden et al). 1990;163:830-3
Hoskins WJ (*see* Rubin et al). 1990;163:69-73
Howseman AM (*see* Johnson et al). 1990;163:601-7
Hsieh H-J (*see* Ho et al). 1990;163:178-88
Hsing AW (*see* Harger et al). 1990;163:130-7
Hsu YC (*see* Romero et al). 1990;163:823-5
Huard P (*see* Garner et al). 1990;163:505-8
Huettner PC (*see* Van Voorhis et al). 1990;163:57-62
Huhta JC (*see* Moise et al). 1990;163:776-84
Humblet DMP (*see* Jandrain et al). 1990;163:378-81 (Symposium)
Hume RF Jr. Cocaine effects on fetal behavioral state. 1990;163:1365 (Letter reply)
——, Bowie JD, McCoy C, Magarelli PC, Gall M, Hertzberg BS, Carroll BA, Killam AP. Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive

value for pregnancy-induced hypertension. 1990;163:826-9
Hümpel M, Täuber U, Kuhnz W, Pfeffer M, Brill K, Biol D, Heithecker R, Louton T, Steinberg B, Seifert W, Schütt B. Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives. 1990;163:329-33 (Symposium)
—— (*see* Täuber et al). 1990;163:1414-20 (Symposium)
Hunse C (*see* Benzaquen et al). 1990;163:484-90
—— (*see* Gagnon et al). 1990;163:1894-9
Hunter VJ, Daly L, Helms M, Soper JT, Berchuck A, Clarke-Pearson DL, Bast RC Jr. The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemotherapy after surgical cytoreduction. 1990;163:1164-7
Hur D (*see* Burke et al). 1990;163:17-8
Hurt WG (*see* Soper et al). 1990;163:1016-23
Hussa RO (*see* Rao et al). 1990;163:1098-9 (Letter)
Husslein PW (*see* Egarter and Husslein). 1990;163:707-8 (Letter reply)
—— (*see* Egarter et al). 1990;163:794-6
Hutson JM (*see* August et al). 1990;163:1612-21
Hvidberg EF. Why do we need pharmacokinetic studies? 1990;163:316-8 (Symposium)

I

Iams JD (*see* Kniss et al). 1990;163:1883-90
Ichihara J (*see* Izumi et al). 1990;163:638-47
Idänpään-Heikkilä J. (*see* Hirvonen and Idänpään-Heikkilä). 1990;163:281-4 (Symposium)
Ikarashi T (*see* Takahashi et al). 1990;163:112-3
Ikeda T, Mori N. Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry. 1990;163:494-8
Ikegami M (*see* Tabor et al). 1990;163:669-76
Imrie SJ (*see* Cameron et al). 1990;163:931-4
Indik JH, Reed KL. Variation and correlation in human fetal umbilical Doppler velocities with fetal breathing: evidence of the cardiac-placental connection. 1990;163:1792-6
Insler V (*see* Glezerman et al). 1990;163:704 (Letter reply)
Irion GL, Clark KE. Relationship between the ovine fetal umbilical artery blood flow waveform and umbilical vascular resistance. 1990;163:222-9
Irion O (*see* Dupont et al). 1990;163:1527-8
Isla M, Dyer DC. Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy. 1990;163:1337-44
Ivanisevic M (*see* Fuchs et al). 1990;163:1961-7
Iwata Y (*see* Lin and Iwata). 1990;163:243-4 (Letter)
Izquierdo LA, Kushnir O, Knieriem K, Wernley JA, Curet LB. Effect of mitral valve prosthetic surgery on the outcome of a growth-retarded fetus: a case report. 1990;163:584-7
Izumi H, Ichihara J, Uchiumi Y, Shirakawa K. Gestational changes in mechanical properties of skinned muscle tissues of human myometrium. 1990;163:638-47

J

Jaakkola U-M (*see* Pulkkinen and Jaakkola). 1990;163:687-8 (Letter reply)
Jackson DN (*see* Lewis et al). 1990;163:773-5
Jaffe RB, Plosker S, Marshall L, Martin MC. Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women. 1990;163:1727-31 (Symposium)
——, Yen SSC. Pulsatile GnRH in clinical medicine: introductory remarks. 1990;163:1719-20 (Symposium)
Jakobi P (*see* Weissman and Jakobi). 1990;163:1716 (Letter)
Jakobovits ÁA. Changes in the incidence of ectopic pregnancy in Hungary. 1990;163:265-6 (Letter)
James DK (*see* Mills et al). 1990;163:12-7
Jaminet CB (*see* Jandrain et al). 1990;163:378-81 (Symposium)
Jandrain BJ, Humblet DMP, Jaminet CB, Scheen AJ, Gaspard UJ, Lefebvre PJ. Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial. 1990;163:378-81 (Symposium)
Jauniaux E, Campbell S. Ultrasonographic assessment of placental abnormalities. 1990;163:1650-8
——, Gibb D, Moscoso G, Campbell S. Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level. 1990;163:1558-60
Jayo MJ, Weaver DS, Adams MR, Rankin SE. Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys. 1990;163:614-8
Jeanty P, Kepple D, Roussis P, Shah D. In utero detection of cardiac failure from an aneurysm of the vein of Galen. 1990;163:50-1
Jedeikin R, Olsfanger D. Safety of 32% dextran 70 for hysteroscopy. 1990;163:2029-30 (Letter reply)
Jenkins S (*see* Figueroa et al). 1990;163:648-54
Jennings RH Jr (*see* Orr et al). 1990;163:1485-9
Jenson AB (*see* Lewandowski et al). 1990;163:1333-7
Jespersen J, Petersen KR, Skouby SO. Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid. 1990;163:396-403 (Symposium)
——, Skouby SO, Kluft C. First European Conference on Sex Steroids and Metabolism: introductory remarks. 1990;163:273 (Symposium)
—— (*see* Gram et al). 1990;163:438-42 (Symposium)
—— (*see* Kooistra et al). 1990;163:404-13 (Symposium)
Jick H (*see* Milunsky et al). 1990;163:693 (Letter reply)
Jimenez C (*see* Romero et al). 1990;163:757-61
—— (*see* Romero et al). 1990;163:968-74
Jobe AH (*see* Tabor et al). 1990;163:669-76
Johnson A (*see* Dennis et al). 1990;163:267 (Letter reply)
—— (*see* McGregor et al). 1990;163:1465-73
Johnson AM, Palomaki GE, Haddow JE. The effect of adjusting maternal serum α-fetoprotein levels for maternal weight in pregnancies with fetal open spina bifida. 1990;163:9-11
Johnson IR, Stehling MK, Blamire AM, Coxon RJ, Howseman AM, Chapman B, Ordidge RJ, Mansfield P, Symonds EM, Worthington BS, Coupland RE. Study of internal structure of the human fetus in utero by echo-planar magnetic resonance imaging. 1990;163:601-7
Johnson JWC, Egerman RS, Moorhead J. Cases with ruptured membranes that "reseal." 1990;163:1024-32
——, Richards DS. Definition of fetal asphyxia. 1990;163:1364 (Letter reply)
Johnson ML (*see* Sollenberger et al). 1990;163:1529-34
Johnson MP, Drugan A, Koppitch FC III, Uhlmann WR, Ev-

ans MI. Postmortem chorionic villus sampling is a better method for cytogenetic evaluation of early fetal loss than culture of abortus material. 1990;163:1505-10
Johnson P, Maxwell DJ. Normal amniotic pressure in oligohydramnios after preterm rupture of membranes. 1990;163:1103-4 (Letter)
—— (*see* Dennis et al). 1990;163:267 (Letter reply)
Johnston JM (*see* McCoshen et al). 1990;163:1632-40
Johnston MM, Sanchez-Ramos L, Vaughn AJ, Todd MW, Benrubi GI. Antibiotic therapy in preterm premature rupture of membranes: a randomized, prospective, double-blind trial. 1990;163:743-7
Johnstone J (*see* Cameron et al). 1990;163:931-4
Jones EE (*see* Seifer and Jones). 1990;163:1715 (Letter)
Jones JS (*see* Arnett et al). 1990;163:1074-80
Juchem M, Pollow K. Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor. 1990;163:2171-83 (Symposium)
Judson FN (*see* McGregor et al). 1990;163:1465-73
—— (*see* McGregor et al). 1990;163:1580-91
Juett D (*see* Mooney et al). 1990;163:30-6
Juhan-Vague I, Vague P. Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease. 1990;163:313-5 (Symposium)
Juneja SC, Dodson MG. In vitro effect of RU 486 on sperm-egg interaction in mice. 1990;163:216-21
Jung-Hoffmann C, Kuhl H. Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism. 1990;163:2183-97 (Symposium)
—— (*see* Kuhl et al). 1990;163:363-9 (Symposium)

K

Kadar N. Measures of test performance and predictive values. 1990;163:1374-5 (Letter)
——. Use of serial human chorionic gonadotropin measurements to differentiate between intrauterine and ectopic pregnancies. 1990;163:259-60 (Letter)
Kafrissen ME (*see* McGuire et al). 1990;163:2127-31 (Symposium)
Kalhan SC, Rossi E, Philipson E. Is meconium aspiration an intrauterine event? 1990;163:250-1 (Letter reply)
Kamiura S (*see* Nobunaga et al). 1990;163:634-8
Kanazawa K (*see* Takahashi et al). 1990;163:112-3
Kaneko M (*see* Koike et al). 1990;163:696 (Letter)
Kanwar AJ, Kaur S. Pemphigus in pregnancy. 1990;163:1097 (Letter reply)
Kao M-S (*see* Mutch et al). 1990;163:1920-4
Kaplan JR (*see* Adams et al). 1990;163:1388-92 (Symposium)
Karchmar EJ (*see* Low et al). 1990;163:1131-5
Kasturi RV (*see* Macri et al). 1990;163:1248-53
—— (*see* Macri et al). 1990;163:1373-4 (Letter reply)
Katz J (*see* Hoskins et al). 1990;163:1944-7
Katz M (*see* Hendricks and Katz). 1990;163:699-700 (Letter)
Katz VL, Thorp JM Jr, Rozas L, Bowes WA Jr. The natural history of thrombocytopenia associated with preeclampsia. 1990;163:1142-3
—— (*see* Watson et al). 1990;163:1456-7
Kauma S, Matt D, Strom S, Eierman D, Turner T. Interleukin-1β, human leukocyte antigen DR1, and transforming growth factor-β expression in endometrium, placenta, and placental membranes. 1990;163:1430-7
Kaunitz AM (*see* Sanchez-Ramos et al). 1990;163:1081-8
Kauppila A (*see* Puistola et al). 1990;163:1276-81
Kaur S (*see* Kanwar and Kaur). 1990;163:1097 (Letter reply)
Keene CL (*see* Saller et al). 1990;163:922-5
Kelleher CC. Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease. 1990;163:392-5 (Symposium)
Keller JD, Metzger BE, Dooley SL, Tamura RK, Sabbagha RE, Freinkel N. Infants of diabetic mothers with accelerated fetal growth by ultrasonography: Are they all alike? 1990;163:893-7
—— (*see* Martin et al). 1990;163:1209-16
Kelly MA (*see* Trimmer et al). 1990;163:1900-3
Kelton JG (*see* Burrows and Kelton). 1990;163:1147-50
Kepple D (*see* Jeanty et al). 1990;163:50-1
Kestler E (*see* Villar et al). 1990;163:151-7
Khalifé S (*see* Villeneuve et al). 1990;163:1641-6
Khan-Dawood FS (*see* Hecht et al). 1990;163:1986-91
Khoury AD (*see* Moretti et al). 1990;163:833-8
—— (*see* Moretti et al). 1990;163:1844-8
Khoury J (*see* Stamler et al). 1990;163:1217-21
Killam AP (*see* Hume et al). 1990;163:826-9
Kilpatrick DC (*see* Liston and Kilpatrick). 1990;163:1365-6 (Letter)
Kim MH (*see* Goldberg et al). 1990;163:65-8
Kimura T (*see* Nobunaga et al). 1990;163:634-8
Kinsky R (*see* Szekeres-Bartho et al). 1990;163:1320-1
Kirk KA (*see* Owen et al). 1990;163:873-9
Kirshon B (*see* Smith et al). 1990;163:98-9
Kitzmiller JL (*see* Major and Kitzmiller). 1990;163:838-44
Kivikoski AI, Martin CM, Smeltzer JS. Transabdominal and transvaginal ultrasonography in the diagnosis of ectopic pregnancy: a comparative study. 1990;163:123-8
——, ——, ——. Transabdominal and transvaginal ultrasonography in the diagnosis of ectopic pregnancy: a comparative study (1990;163:123-9). 1990;163:2030 (Correction)
Kjæaer A (*see* Lebech et al). 1990;163:414-6 (Symposium)
—— (*see* Refn et al). 1990;163:374-7 (Symposium)
Kjos SL, Buchanan TA, Greenspoon JS, Montoro M, Bernstein GS, Mestman JH. Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months post partum. 1990;163:93-8
——, Shoupe D, Douyan S, Friedman RL, Bernstein GS, Mestman JH, Mishell DR Jr. Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: results of a controlled, randomized, prospective study. 1990;163:1822-7
——, Walther FJ, Montoro M, Paul RH, Diaz F, Stabler M. Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests. 1990;163:898-903
Klebanoff MA, Shiono PH, Carey JC. The effect of physical activity during pregnancy on preterm delivery and birth weight. 1990;163:1450-6
Kloosterboer HJ, Rekers H. Effects of three combined oral contraceptive preparations containing desogestrel plus ethinyl estradiol on lipid metabolism in comparison with two levonorgestrel preparations. 1990;163:370-3 (Symposium)
Kloosterboer L (*see* Bergink et al). 1990;163:2132-7 (Symposium)
Kluft C. Disorders of the hemostatic system and the risk of the development of thrombotic and cardiovascular diseases: limitations of laboratory diagnosis. 1990;163:305-12 (Symposium)
—— (*see* Jespersen et al). 1990;163:273 (Symposium)
—— (*see* Kooistra et al). 1990;163:404-13 (Symposium)
Knape KG (*see* Downing and Knape). 1990;163:1096-7 (Letter)

Knieriem K (*see* Izquierdo et al). 1990;163:584-7
Knight GJ (*see* Haddow et al). 1990;163:1372 (Letter)
Kniss DA, Mershon J, Su H-C, Sonek J, Fertel RH, Waxman M, Iams JD, Gabbe SG. Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells. 1990;163:1883-90
Knobil E. The GnRH pulse generator. 1990;163:1721-6 (Symposium)
Knoll K (*see* Harstad et al). 1990;163:912-6
Knox GE (*see* Harger et al). 1990;163:130-7
Knuppel RA (*see* O'Brien et al). 1990;163:1796-8
Knuppen R (*see* Ball and Knuppen). 1990;163:2163-70 (Symposium)
Koenigsberg M (*see* Chazotte et al). 1990;163:1180-1
Kofinas AD (*see* Hatjis et al). 1990;163:45-50
Koga Y, Mizumoto M, Matsumoto Y, Hattori T, Tanaka S, Tanaka T, Fujimoto S. Prenatal diagnosis of fetal intracranial calcifications. 1990;163:1543-5
Koike H, Chuganji Y, Watanabe H, Kaneko M, Noda S-i, Mori N. Conservative treatment of ovarian pregnancy by local prostaglandin $F_{2\alpha}$ injection. 1990;163:696 (Letter)
Kooistra T, Bosma PJ, Jespersen J, Kluft C. Studies on the mechanism of action of oral contraceptives with regard to fibrinolytic variables. 1990;163:404-13 (Symposium)
Koonings PP (*see* Bergman et al). 1990;163:2025-6 (Letter reply)
Kopelman JN (*see* Christian et al). 1990;163:848-55
Koppitch FC III (*see* Johnson et al). 1990;163:1505-10
Koren G. Perception of teratogenic risk by pregnant women exposed to diagnostic radiation during pregnancy. 1990;163:695-6 (Letter reply)
Korn WT, Schatzki SC, DiSciullo AJ, Scully RE. Papillary cystadenoma of the broad ligament in von Hippel-Lindau disease. 1990;163:596-8
Koutsilieris M, Michaud J, Nikolis A. Preferential mitogenic activity for myoblast-like cells can be extracted from uterine leiomyoma tissues. 1990;163:1665-70
Koyanagi T (*see* Horimoto et al). 1990;163:1480-4
Krantz DA (*see* Macri et al). 1990;163:1248-53
—— (*see* Macri et al). 1990;163:1373-4 (Letter reply)
Kratzer PG, Taylor RN. Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels. 1990;163:1497-502
Krebs D (*see* Mallmann et al). 1990;163:1367 (Letter)
Krebs H-B (*see* Helmkamp and Krebs). 1990;163:1554-7
Kredentser JV (*see* McCoshen et al). 1990;163:1632-40
Kreitzer L (*see* Copel et al). 1990;163:751-6
Kretchmer N (*see* Green et al). 1990;163:86-92
Kühl C (*see* Skouby et al). 1990;163:343-8 (Symposium)
Kuhl H. Pharmacokinetics of oral contraceptive steroids and drug interaction: conclusion. 1990;163:2216-8 (Symposium)
——. Pharmacokinetics of oral contraceptive steroids and drug interaction: introduction. 1990;163:2113 (Symposium)
——, März W, Jung-Hoffmann C, Heidt F, Gross W. Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives. 1990;163:363-9 (Symposium)
—— (*see* Jung-Hoffmann and Kuhl). 1990;163:2183-97 (Symposium)
Kuhlmann RS, Werner AL, Abramowicz J, Warsof SL, Arrington J, Levy DL. Placental histology in fetuses between 18 and 23 weeks' gestation with abnormal karyotype. 1990;163:1264-70
Kuhnz W. Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women. 1990;163:2120-7 (Symposium)
—— (*see* Hümpel et al). 1990;163:329-33 (Symposium)
—— (*see* Täuber et al). 1990;163:1414-20 (Symposium)
Kunz F, Pechlaner C, Tabarelli M, Sölder E, Zwierzina W-D. Influence of oral contraceptives on coagulation tests in native blood and plasma. 1990;163:417-20 (Symposium)
Kunze E (*see* Vasquez et al). 1990;163:2006-12
Kushnir O (*see* Izquierdo et al). 1990;163:584-7
Kutteh WH, Baker VV, Doellgast GJ. Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions. III. Cytotoxic potential in vitro and characterization of antigen(s). 1990;163:1301-6
Kuyper W (*see* Andres et al). 1990;163:935-8

L

Laatikainen T. Corticotropin-releasing hormone in amniotic fluid. 1990;163:1102 (Letter)
Lacarra M (*see* Levin et al). 1990;163:543-4
Lafferty CM (*see* Gilbert et al). 1990;163:998-9
Laippala PJ (*see* Mäkinen et al). 1990;163:266 (Letter reply)
Lancaster WD (*see* Lewandowski et al). 1990;163:1333-7
Landesman SH (*see* Hoegsberg et al). 1990;163:1135-9
—— (*see* Minkoff et al). 1990;163:1598-604
Langer O. Findings not new. 1990;163:2028 (Letter reply)
—— (*see* Berkus et al). 1990;163:1812-7
—— (*see* Berkus et al). 1990;163:1818-21
Langer R, Golan A, Neuman M, Schneider D, Bukovsky I, Caspi E. The effect of large uterine fibroids on urinary bladder function symptoms. 1990;163:1139-41
Lao T. Maternal serum α-fetoprotein level useful screening test. 1990;163:240 (Letter)
LaPolla JP, Nicosia S, McCurdy C, Songster C, Ruffolo E, Roberts WS, Hoffman MS, Fiorica JV, Cavanagh D. Experience with the EndoPap device for the cytologic detection of uterine cancer and its precursors: a comparison of the EndoPap with fractional curettage or hysterectomy. 1990;163:1055-60
—— (*see* Cavanagh et al). 1990;163:1007-15
La Puma J, Schiedermayer DL, Grover JL. Surrogate parenting contracts. 1990;163:680 (Letter reply)
Laragh JH (*see* August et al). 1990;163:1612-21
Laros RK Jr (*see* Combs et al). 1990;163:100-4
Larsen JW Jr (*see* Macri et al). 1990;163:1248-53
—— (*see* Macri et al). 1990;163:1373-4 (Letter reply)
LaSala A (*see* Berkowitz and LaSala). 1990;163:981-5
Lavy G (*see* Grifo et al). 1990;163:2013-9
Lazebnik N, Hendrix PV, Ashmead GG, Ashmead JW, Mann LI. Detection of fetal blood contamination by amniotic fluid obtained during cordocentesis. 1990;163:78-80
Lazzari M (*see* Riveros et al). 1990;163:704-5 (Letter)
Lebech A-M, Kjæaer A, Lebech PE. Metabolic changes during the normal menstrual cycle: a longitudinal study. 1990;163:414-6 (Symposium)
—— (*see* Refn et al). 1990;163:374-7 (Symposium)
Lebech PE (*see* Lebech et al). 1990;163:414-6 (Symposium)
Lebo RV (*see* Pinckert et al). 1990;163:684 (Letter reply)
Lechner W. Measurement of cardiac output with impedance cardiography. 1990;163:1096 (Letter)
Ledermüller G (*see* Loewit et al). 1990;163:708-10 (Letter)
Lee D (*see* McCoshen et al). 1990;163:81-5
Lee T-Y (*see* Ho et al). 1990;163:178-88
Lee W. Ultrasonographic guidance for central venous access. 1990;163:252 (Letter reply)

Lefebvre PJ (*see* Gaspard and Lefebvre). 1990;163:334-43 (Symposium)
—— (*see* Jandrain et al). 1990;163:378-81 (Symposium)
LeFevre M (*see* Ewigman et al). 1990;163:256-7 (Letter)
Leiberman JR, van Vroonhoven CCJ, Beckmann I, van der Kwast TH, Wallenburg HCS. Uterine artery estrogen receptors in the nonpregnant and pregnant guinea pig. 1990;163:1685-8
Leland MM (*see* Hankins et al). 1990;163:728-32
Lench JB (*see* Gambone et al). 1990;163:545-50
Lenfant C, Gifford RW Jr, Zuspan F. National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy: foreword. 1990;163:2113 (Symposium)
Lenke RR, Persutte WH, Nemes J. Ultrasonographic assessment of intestinal damage in fetuses with gastroschisis: Is it of clinical value? 1990;163:995-8
Lenox JW, Uguru V, Cibils LA. Effects of hypertension on pregnancy monitoring and results. 1990;163:1173-9
Lenz T (*see* August et al). 1990;163:1612-21
Leonardi G (*see* Baudo et al). 1990;163:1274-5
Lepot MR (*see* David et al). 1990;163:420-3 (Symposium)
Letsky EA (*see* Nicolini et al). 1990;163:1144-6
Letterie GS, Bruner JP, Sarno AP Jr. A convenient method to identify prior surgical procedures. 1990;163:1100 (Letter)
Leung PCK (*see* Rodway et al). 1990;163:1546-51
Leveno KJ (*see* Cox et al). 1990;163:767-72
—— (*see* Trimmer et al). 1990;163:1900-3
Levi A (*see* Meizner et al). 1990;163:128-9
Levin JH, Lacarra M, d'Ablaing G, Grimes DA, Vermesh M. Mifepristone (RU 486) failure in an ovarian heterotopic pregnancy. 1990;163:543-4
——, Tonetta SA, Lobo RA. Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells. 1990;163:1932-8
Levy A (*see* Meizner et al). 1990;163:267-8 (Letter)
Levy DL (*see* Kuhlmann et al). 1990;163:1264-70
Lewandowski G, Delgado G, Holloway RW, Farrell M, Jenson AB, Lancaster WD. The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes. 1990;163:1333-7
Lewis DF, Towers CV, Garite TJ, Jackson DN, Nageotte MP, Major CA. Fetal gastroschisis and omphalocele: Is cesarean section the best mode of delivery? 1990;163:773-5
—— (*see* Harding et al). 1990;163:1566-8
Lewis JL Jr (*see* Rubin et al). 1990;163:69-73
Lin B-L, Iwata Y. Modified laparoscopy for monitoring transcervical surgery. 1990;163:243-4 (Letter)
Lindblom B, Hahlin M, Sjöblom P. Caution in use of new test for ectopic pregnancy. 1990;163:694-5 (Letter reply)
——, ——, ——. Use of serial human chorionic gonadotropin measurements to differentiate between intrauterine and ectopic pregnancies. 1990;163:260-1 (Letter reply)
Liner RI. Intrauterine *Listeria* infection: prenatal diagnosis by biophysical assessment and amniocentesis. 1990;163:1596-7
Lipper EG, Ross GS, Auld PAM, Glassman MB. Survival and outcome of infants weighing 800 grams at birth 1990;163:146-50
Lippert TH (*see* Dietl and Lippert). 1990;163:707 (Letter)
Liston WA, Kilpatrick DC. Preeclampsia—An endothelial cell disorder plus "something else"? 1990;163:1365-6 (Letter)
Little BB (*see* Harstad et al). 1990;163:912-6
—— (*see* Roe et al). 1990;163:715-8
Liu JH. Hypothalamic amenorrhea: clinical perspectives, pathophysiology, and management. 1990;163:1732-6 (Symposium)
Liu K (*see* Martin et al). 1990;163:1209-16
Livengood CH III, Thomason JL, Hill GB. Bacterial vaginosis: diagnostic and pathogenetic findings during topical clindamycin therapy. 1990;163:515-20
Ljungström K-G. Safety of 32% dextran 70 for hysteroscopy. 1990;163:2029 (Letter)
Lloyd KO (*see* Rubin et al). 1990;163:69-73
Lobe T (*see* Moretti et al). 1990;163:833-8
Lobel SM (*see* Friedman et al). 1990;163:1114-9 (Clin. opinion)
Lobo RA (*see* Cassidenti et al). 1990;163:1953-60
—— (*see* Levin et al). 1990;163:1932-8
—— (*see* Paulson et al). 1990;163:2020-3
—— (*see* Shoupe et al). 1990;163:1982-5
Lockwood CJ. Laboratory identification of the lupus anticoagulant in normal pregnancy and pregnancy-induced hypertension. 1990;163:705 (Letter reply)
Loewit K, Hoppichler F, Ledermüller G, Widhalm R. Ovulation prediction from cyclic changes in salivary electrical conductivity. 1990;163:708-10 (Letter)
Loffer FD. Explanation of mechanism of Hyskon solution reaction needed. 1990;163:2029 (Letter)
——. Fluid cooling of artificial sapphire tips of laser. 1990;163:681 (Letter)
Lohda AK (*see* Romero et al). 1990;163:968-74
Long CA, Bauer GS, Lowe ME, Strauss AW, Gast MJ. Isolation and characterization of the gene from a human genome encoding 17β-estradiol dehydrogenase: a comparison of Jar and BeWo choriocarcinoma cell lines. 1990;163:1976-81
Lorber DL (*see* Tibaldi et al). 1990;163:509-10
Louton T (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Louv WC (*see* Barbone et al). 1990;163:510-4
Low JA, Muir DW, Pater EA, Karchmar EJ. The association of intrapartum asphyxia in the mature fetus with newborn behavior. 1990;163:1131-5
Lowe DG (*see* van Dam et al). 1990;163:698-9 (Letter)
Lowe ME (*see* Long et al). 1990;163:1976-81
Lowe TW (*see* Yeomans et al). 1990;163:958-64
Lowery CL Jr (*see* Hankins et al). 1990;163:728-32
Lowther GW (*see* Cameron et al). 1990;163:931-4
Lucifora G, Martines F, Calabro S, Carroccio G, Brigandi A, de Pasquale R. HBcAg identification in the placental cytotypes of symptom-free HBsAg-carrier mothers: a study with the immunoperoxidase method. 1990;163:235-9
Luthy DA (*see* Mahony et al). 1990;163:1513-22

M

Mabie WC, Ahokas RA, Sibai BM. Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat. 1990;163:1861-7
—— (*see* Barton et al). 1990;163:534-8
MacGregor SN (*see* Silver et al). 1990;163:917-22
—— (*see* Utter et al). 1990;163:1781-4
Mackall JA (*see* Mader et al). 1990;163:1578-9
MacKenzie JRM (*see* Cameron et al). 1990;163:931-4
Macnaughton M. Ethics of new reproductive technologies. 1990;163:1376 (Letter reply)
Macri JN, Kasturi RV, Krantz DA, Cook EJ, Moore ND, Young JA, Romero K, Larsen JW Jr. Maternal serum Down syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin. 1990;163:1248-53
——, ——, ——, ——, Sunderji SG, Larsen JW Jr. Maternal

serum unconjugated estriol levels are lower in the presence of fetal Down syndrome. 1990;163:1373-4 (Letter reply)
Madden S (*see* Back et al). 1990;163:2138-45 (Symposium)
Mader SL, Mackall JA, Emert MP. Hyperadrenergic orthostatic intolerance as a result of postpartum blood loss. 1990;163:1578-9
Magarelli PC (*see* Hume et al). 1990;163:826-9
Magargee SF (*see* Vasquez et al). 1990;163:2006-12
Magness RR (*see* Brown et al). 1990;163:1853-61
Mahony BS, Petty CN, Nyborg DA, Luthy DA, Hickok DE, Hirsch JH. The stuck twin phenomenon: ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses. 1990;163:1513-22
Maier D. Association between autoantibodies and unexplained infertility unproved. 1990;163:243 (Letter)
Major CA, Kitzmiller JL. Perinatal survival with expectant management of midtrimester rupture of membranes. 1990;163:838-44
—— (*see* Harding et al). 1990;163:1566-8
—— (*see* Lewis et al). 1990;163:773-5
—— (*see* Stern et al). 1990;163:176-7
Mäkinen JI, Erkkola RU, Laippala PJ. Changes in the incidence of ectopic pregnancy in Hungary. 1990;163:266 (Letter reply)
Mallmann P, Werner A, Krebs D. Serum levels of interleukin-2 and tumor necrosis factor α in women with recurrent abortion. 1990;163:1367 (Letter)
Manabe Y, Yoshida Y. Morphologic study of changes of collagenous tissue in the amnion and cervix during prostaglandin-induced abortion and delivery. 1990;163:52-6
Mann LI (*see* Lazebnik et al). 1990;163:78-80
Mann WJ. A comment on thumbtacks for hemorrhage control. 1990;163:1092-3 (Letter)
Mansfield P (*see* Johnson et al). 1990;163:601-7
Mantingh A (*see* van Loon et al). 1990;163:1256-60
Marcoux S (*see* Villeneuve et al). 1990;163:1641-6
Margara R (*see* Dimitry et al). 1990;163:244-5 (Letter)
Mari G (*see* Moise et al). 1990;163:776-84
—— (*see* Smith et al). 1990;163:567-9
Maria B (*see* Cabrol et al). 1990;163:540-2
Mariani G (*see* Abbate et al). 1990;163:424-9 (Symposium)
Markman M (*see* Rubin et al). 1990;163:69-73
Markoff E (*see* McCoshen et al). 1990;163:81-5
Marquette GP, Boucher M, Desrochers M, Dallaire L. Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femur length ratio. 1990;163:1604-5
Mar[brev]sál K (*see* Stale et al). 1990;163:26-9
Marshall L (*see* Jaffe et al). 1990;163:1727-31 (Symposium)
Martens M, Faro S. Cefotetan and the lack of associated bleeding. 1990;163:251 (Letter)
Martin AO, Dempsey LM, Minogue J, Liu K, Keller J, Tamura R, Freinkel N. Maternal serum α-fetoprotein levels in pregnancies complicated by diabetes: implications for screening programs. 1990;163:1209-16
Martin CM (*see* Kivikoski et al). 1990;163:123-8
Martin MC (*see* Jaffe et al). 1990;163:1727-31 (Symposium)
Martines F (*see* Lucifora et al). 1990;163:235-9
Martinez-Mir I, Estañ L, Morales-Olivas FJ, Rubio E. Studies of the spontaneous motility and the effect of histamine on isolated myometrial strips of the nonpregnant human uterus: the influence of various uterine abnormalities. 1990;163:189-95
Martinez-Schnell B (*see* Sanchez-Ramos et al). 1990;163:1081-8
März W (*see* Kuhl et al). 1990;163:363-9 (Symposium)
Mascioli SR (*see* Cauley et al). 1990;163:1438-44
Mason B (*see* Gonsoulin et al). 1990;163:1172-3
Mason RA (*see* Cunningham et al). 1990;163:453-9
Massad LS (*see* Mutch et al). 1990;163:1920-4
Mastroiacovo P, Zampino G, Valente M. Perception of teratogenic risk by pregnant women exposed to diagnostic radiation during pregnancy. 1990;163:695 (Letter)
Matera C, Warren WB, Moomjy M, Fink DJ, Fox HE. Prevalence of use of cocaine and other substances in an obstetric population. 1990;163:797-801
Mathers AM (*see* Cameron et al). 1990;163:931-4
Mathur S (*see* Daru and Mathur). 1990;163:1622-9
Matsumoto AM, Gross KM, Sheckter CB, Bremner WJ. The luteinizing hormone–releasing hormone pulse generator in men: abnormalities and clinical management. 1990;163:1743-51 (Symposium)
Matsumoto K (*see* Sakata et al). 1990;163:1679-84
Matsumoto Y (*see* Koga et al). 1990;163:1543-5
Matt DW (*see* Kauma et al). 1990;163:1430-7
—— (*see* Nestler et al). 1990;163:1243-6
Mattsson L-[oc]A (*see* Samsioe and Mattsson). 1990;163:354-8 (Symposium)
Mauri M (*see* Baudo et al). 1990;163:1274-5
Maxwell DJ (*see* Johnson and Maxwell). 1990;163:1103-4 (Letter)
Mazor M (*see* Romero et al). 1990;163:757-61
—— (*see* Romero et al). 1990;163:823-5
—— (*see* Romero et al). 1990;163:968-74
McAlpin-Cadkin J (*see* Cadkin and McAlpin-Cadkin). 1990;163:1103 (Letter)
McCaffrey R (*see* Berkowitz and McCaffrey). 1990;163:1200-1
McCalla S (*see* Minkoff et al). 1990;163:521-6
McColgin SW, Williams LM, Sorrells TL, Morrison JC. Hemoperitoneum as a result of coital injury without associated vaginal injury. 1990;163:1503-5
McCormack WM (*see* Covino and McCormack). 1990;163:116-8
McCoshen JA, Hoffman DR, Kredentser JV, Araneda C, Johnston JM. The role of fetal membranes in regulating production, transport, and metabolism of prostaglandin E_2 during labor. 1990;163:1632-40
——, Patalas E, Markoff E, Lee D, Ming Y. Comparisons of glycosylated and nonglycosylated amniotic fluid prolactins in normal second-and third-trimester pregnancies. 1990;163:81-5
McCoy C (*see* Hume et al). 1990;163:826-9
McCullough LB (*see* Chervenak and McCullough). 1990;163:1425-9 (Clin. opinion)
—— (*see* Chervenak et al). 1990;163:257-8 (Letter reply)
—— (*see* Chervenak et al). 1990;163:688-9 (Letter reply)
McCurdy C (*see* LaPolla et al). 1990;163:1055-60
McDonough PG (*see* Tho et al). 1990;163:1968-75
McFarland KF (*see* Smythe et al). 1990;163:1037-9
McFee J (*see* McGregor et al). 1990;163:1580-91
McGregor JA, French JI, Richter R, Franco-Buff A, Johnson A, Hillier S, Judson FN, Todd JK. Antenatal microbiologic and maternal risk factors associated with prematurity. 1990;163:1465-73
——, ——, ——, Vuchetich M, Bachus V, Seo K, Hillier S, Judson FN, McFee J, Schoonmaker J, Todd JK. Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment. 1990;163:1580-91
McGuire JL, Phillips A, Hahn D, Tolman EL, Flor S, Kafrissen

ME. Pharmacologic and pharmacokinetic characteristics of norgestimate and its metabolites. 1990;163:2127-31 (Symposium)
McLaren RA (*see* Saving et al). 1990;163:1369-70 (Letter)
McLelland R (*see* Dodd and McLelland). 1990;163:689-90 (Letter)
McLeod AGW. Retrospection and prospection. 1990;163: 1000-6 (Pres. address)
McMenamy K (*see* Devaskar et al). 1990;163:1350-6
McNellis D (*see* Ewigman et al). 1990;163:256-7 (Letter)
Mead PB (*see* Harger et al). 1990;163:130-7
Medearis AL (*see* Carlson et al). 1990;163:1785-7
Meis PJ (*see* Cuthrell et al). 1990;163:170-4
Meizner I, Levi A, Carmi R, Robinsin C. Niemann-Pick disease associated with nonimmune hydrops fetalis. 1990;163:128-9
——, Levy A, Cohen J. Spontaneous resolution of a nuchal fetal cystic hygroma diagnosed early in the second trimester of pregnancy. 1990;163:267-8 (Letter)
Menczer J (*see* Baruch and Menczer). 1990;163:703-4 (Letter)
—— (*see* Schiff et al). 1990;163:1375 (Letter reply)
Mendez H (*see* Minkoff et al). 1990;163:1598-604
Meredith WD (*see* Smythe et al). 1990;163:1037-9
Meriggiola MC (*see* Filicori et al). 1990;163:1737-42 (Symposium)
Merlob P (*see* Metzker and Merlob). 1990;163:1097 (Letter)
Mershon J (*see* Kniss et al). 1990;163:1883-90
Merzbach D (*see* Goldstein et al). 1990;163:1261-3
Mestman JH (*see* Kjos et al). 1990;163:93-8
—— (*see* Kjos et al). 1990;163:1822-7
Metzger BE (*see* Keller et al). 1990;163:893-7
—— (*see* Rizzo et al). 1990;163:1458-64
Metzker A, Merlob P. Pemphigus in pregnancy. 1990;163:1097 (Letter)
Meyer BA (*see* Dickinson et al). 1990;163:1831-5
Meyers CM (*see* Shulman et al). 1990;163:1606-9
Meyers RL (*see* Paulick et al). 1990;163:1357-63
Michaud J (*see* Koutsilieris et al). 1990;163:1665-70
Michelacci L (*see* Filicori et al). 1990;163:1737-42 (Symposium)
Mikhail MS (*see* Basu et al). 1990;163:1948-52
Millard DD (*see* Utter et al). 1990;163:1781-4
Miller FC (*see* Hardardottir et al). 1990;163:1925-31
Miller G (*see* Suzuki and Miller). 1990;163:1103 (Letter)
Miller RC (*see* Watson et al). 1990;163:1456-7
Miller TC (*see* Saving et al). 1990;163:1369-70 (Letter)
Milligan JE. Credit neonatal or obstetric care? 1990;163:1716-7 (Letter reply)
Mills MD, Nageotte MP, Elliott JP, Crade M, Dorchester W. Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants. 1990;163:1568-74
Mills MS, James DK, Slade S. Two-tier approach to biophysical assessment of the fetus. 1990;163:12-7
Milunsky A, Jick H, Willett W. Maternal serum α-fetoprotein in pregnancy. 1990;163:693 (Letter reply)
Mimouni F (*see* Stamler et al). 1990;163:1217-21
Ming Y (*see* McCoshen et al). 1990;163:81-5
Minhas BS (*see* Roudebush et al). 1990;163:1670-3
Minkoff HL, Henderson C, Mendez H, Gail MH, Holman S, Willoughby A, Goedett JJ, Rubinstein A, Stratton P, Walsh JH, Landesman SH. Pregnancy outcomes among mothers infected with human immunodeficiency virus and uninfected control subjects. 1990;163:1598-604
——, McCalla S, Delke I, Stevens R, Salwen M, Feldman J. The relationship of cocaine use to syphilis and human immunodeficiency virus infections among inner city parturient women. 1990;163:521-6
——, Moreno JL. Drug prophylaxis for human immunodeficiency virus infected pregnant women: ethical considerations. 1990;163:1111-4 (Clin. opinion)
—— (*see* Hoegsberg et al). 1990;163:1135-9
Minogue J (*see* Martin et al). 1990;163:1209-16
Miodovnik M (*see* Stamler et al). 1990;163:1217-21
Mishell DR Jr (*see* Kjos et al). 1990;163:1822-7
—— (*see* Shoupe et al). 1990;163:1982-5
Mitchell BD (*see* Berkus et al). 1990;163:1812-7
—— (*see* Berkus et al). 1990;163:1818-21
Mitchell JM (*see* Roberts and Mitchell). 1990;163:1560-6
Mitchell MD (*see* Branch et al). 1990;163:210-6
—— (*see* Dudley et al). 1990;163:1890-3
Mizumoto M (*see* Koga et al). 1990;163:1543-5
Mizutani T (*see* Sakata et al). 1990;163:1679-84
Moawad AH (*see* Pridjian and Moawad). 1990;163:246 (Letter reply)
Moise KJ Jr, Mari G, Fisher DJ, Huhta JC, Cano LE, Carpenter RJ Jr. Acute fetal hemodynamic alterations after intrauterine transfusion for treatment of severe red cell alloimmunization. 1990;163:776-84
—— (*see* Gonsoulin et al). 1990;163:526-7
Mollick J (*see* Chatwani et al). 1990;163:240 (Letter)
Mølsted-Pedersen L (*see* Damm and Mølsted-Pedersen). 1990;163:682-3 (Letter reply)
Mølsted-Pedersen L (*see* Skouby et al). 1990;163:343-8 (Symposium)
Montoro M (*see* Kjos et al). 1990;163:93-8
—— (*see* Kjos et al). 1990;163:898-903
Moodley J (*see* Cabrol et al). 1990;163:540-2
Moomjy M (*see* Matera et al). 1990;163:797-801
Mooney P, Dalton KJ, Swindells HE, Rushant S, Cartwright W, Juett D. Blood pressure measured telemetrically from home throughout pregnancy. 1990;163:30-6
Moore JG (*see* Gambone et al). 1990;163:545-50
Moore ND (*see* Macri et al). 1990;163:1248-53
Moore TR. Superiority of the four-quadrant sum over the single-deepest-pocket technique in ultrasonographic identification of abnormal amniotic fluid volumes. 1990;163:762-7
——, Gale S, Benirschke K. Perinatal outcome of forty-nine pregnancies complicated by acardiac twinning. 1990;163:907-12
——, Piacquadio K. Study results vary in count-to-10 method of fetal movement screening. 1990;163:264-5 (Letter reply)
Moorhead J (*see* Johnson et al). 1990;163:1024-32
Mooyaart EL (*see* van Loon et al). 1990;163:1256-60
Morales-Olivas FJ (*see* Martinez-Mir et al). 1990;163:189-95
Moran O (*see* Schiff et al). 1990;163:1375 (Letter reply)
Moreno JL (*see* Minkoff and Moreno). 1990;163:1111-4 (Clin. opinion)
Moretti MM, Fairlie FM, Akl S, Khoury AD, Sibai BM. The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term. 1990;163:1844-8
——, Khoury A, Rodriquez J, Lobe T, Shaver D, Sibai B. The effect of mode of delivery on the perinatal outcome in fetuses with abdominal wall defects. 1990;163:833-8
—— (*see* Sibai et al). 1990;163:733-8
Mori N (*see* Ikeda and Mori). 1990;163:494-8
—— (*see* Koike et al). 1990;163:696 (Letter)
Morris HA (*see* Nordin et al). 1990;163:140-5
Morrison JC (*see* McColgin et al). 1990;163:1503-5

Morrow RJ, Adamson SL, Bull SB, Ritchie JWK. Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep. 1990;163:1313-20
Morrow WR (*see* Hankins et al). 1990;163:728-32
Mortimer RH (*see* Yap et al). 1990;163:477-8
Moscoso G (*see* Jauniaux et al). 1990;163:1558-60
Mueller RA (*see* Thorp et al). 1990;163:655-6
Muir DW (*see* Low et al). 1990;163:1131-5
Muise K (*see* Ridgway et al). 1990;163:879-82
Müller FB (*see* August et al). 1990;163:1612-21
Munkvad S (*see* Gram et al). 1990;163:438-42 (Symposium)
Murphy A (*see* Xu et al). 1990;163:1493-7
Murray C (*see* Devoe et al). 1990;163:1156-61
Mutch DG, Massad LS, Kao M-S, Collins JL. Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies. 1990;163:1920-4
Mutch L (*see* Dennis et al). 1990;163:267 (Letter reply)
Myatt L (*see* Bennett et al). 1990;163:241-2 (Letter)
Myers SA (*see* Ferguson and Myers). 1990;163:1222-30

N

Nachtigall L (*see* Goldstein et al). 1990;163:119-23
Nachtigall M (*see* Goldstein et al). 1990;163:119-23
Naessens A (*see* Foulon et al). 1990;163:1511-3
Nagamani M, Stuart CA. Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis. 1990;163:1992-7
Nageotte MP (*see* Asrat et al). 1990;163:887-9
—— (*see* Briggs et al). 1990;163:251-2 (Letter reply)
—— (*see* Harding et al). 1990;163:1566-8
—— (*see* Lewis et al). 1990;163:773-5
—— (*see* Mills et al). 1990;163:1568-74
—— (*see* Steiger and Nageotte). 1990;163:808-12
—— (*see* Towers et al). 1990;163:965-7
Nagey DA. Appropriate screening tests. 1990;163:688 (Letter)
——. Can shoulder dystocia be prevented? 1990;163:1095 (Letter)
Nakano H (*see* Horimoto et al). 1990;163:1480-4
Nakayama DK. Survival in a fetus with sacrococcygeal teratoma and hydrops. 1990;163:682 (Letter)
Nathanielsz PW (*see* Copel et al). 1990;163:751-6
—— (*see* Figueroa et al). 1990;163:648-54
Naz RK, Chaudhry A, Witkin SS. Lymphocyte proliferative response to fertilization antigen in patients with antisperm antibodies. 1990;163:610-3
Need AG (*see* Nordin et al). 1990;163:140-5
Neilson JP. Twin-to-twin transfusion syndrome. 1990;163:677-8 (Letter reply)
Nelson GH. Amniotic fluid midtrimester delta optical density measurements at 450 nm in Rh disease. 1990;163:710-1 (Letter)
Nemes J (*see* Lenke et al). 1990;163:995-8
Nerenberg A (*see* Tibaldi et al). 1990;163:509-10
Nervetti G (*see* Rovati et al). 1990;163:1490-2
Ness PM (*see* Witter et al). 1990;163:784-6
Nestler JE, Singh R, Matt DW, Clore JN, Blackard WG. Suppression of serum insulin level by diazoxide does not alter serum testosterone of sex hormone–binding globulin levels in healthy, nonobese women. 1990;163:1243-6
Neuman M (*see* Langer et al). 1990;163:1139-41
Newton ER (*see* Berkus et al). 1990;163:1812-7
—— (*see* Ridgway et al). 1990;163:879-82
Neymeyer-Leloux AN (*see* Gevers Leuven et al). 1990;163:358-62 (Symposium)
—— (*see* Gevers Leuven et al). 1990;163:1410-3 (Symposium)
Nicol SL (*see* Witter et al). 1990;163:784-6
Nicolaides KH (*see* Ribbert et al). 1990;163:569-71
—— (*see* Rizzo et al). 1990;163:1231-8
Nicolini U, Fisk NM, Rodeck CH. Normal amniotic pressure in oligohydramnios after preterm rupture of membranes. 1990;163:1104 (Letter reply)
——, Tannirandorn Y, Gonzalez P, Fisk NM, Beecham J, Letsky EA, Rodeck CH. Continuing controversy in alloimmune thrombocytopenia: fetal hyperimmunoglobulinemia fails to prevent thrombocytopenia. 1990;163:1144-6
Nicosia S (*see* LaPolla et al). 1990;163:1055-60
Nielsen OH (*see* Beck-Nielsen et al). 1990;163:292-5 (Symposium)
Nikolis A (*see* Koutsilieris et al). 1990;163:1665-70
Nobunaga T, Azuma C, Kimura T, Tokugawa Y, Takemura M, Kamiura S, Saji F, Tanizawa O. Differential diagnosis between complete mole and hydropic abortus by deoxyribonucleic acid fingerprints. 1990;163:634-8
Noda S-i (*see* Koike et al). 1990;163:696 (Letter)
Nordin BEC, Morris HA, Need AG, Horowitz M, Robertson WG. Relationship between plasma calcium fractions, other bone-related variables, and serum follicle-stimulating hormone levels in premenopausal, perimenopausal, and postmenopausal women. 1990;163:140-5
Nores J (*see* Romero et al). 1990;163:757-61
—— (*see* Romero et al). 1990;163:823-5
—— (*see* Romero et al). 1990;163:968-74
Nosari AM (*see* Baudo et al). 1990;163:1274-5
Nozawa S. Flow cytometric quantitation of tumor-associated antigens in solid tumors. 1990;163:699 (Letter reply)
Nsieh R-P (*see* Ho et al). 1990;163:178-88
Nugent CE (*see* Andersen et al). 1990;163:859-67
Nyborg DA (*see* Mahony et al). 1990;163:1513-22

O

O'Brien TJ (*see* Hardardottir et al). 1990;163:1925-31
O'Brien WF, Knuppel RA, Torres C, Sternlicht D. Potential prenatal predictions of Down syndrome: a statistical analysis. 1990;163:1796-8
—— (*see* Carlan et al). 1990;163:490-3
O'Connor M (*see* Saving et al). 1990;163:1369-70 (Letter)
Odom LD, Plouffe L Jr, Butler WJ. 5-Fluorouracil exposure during the period of conception: report on two cases. 1990;163:76-7
Oetomo SB (*see* Tabor et al). 1990;163:669-76
Ojomo EO, Coustan DR. Absence of evidence of pulmonary maturity at amniocentesis in term infants of diabetic mothers. 1990;163:954-7
O'Kell RT (*see* Thorp et al). 1990;163:607-9
O'Leary JA. Can shoulder dystocia be predicted? 1990;163:680 (Letter reply)
——. Can shoulder dystocia be prevented? 1990;163:1095-6 (Letter reply)
Olsfanger D (*see* Jedeikin and Olsfanger). 1990;163:2029-30 (Letter reply)
Olson DR (*see* Saftlas et al). 1990;163:460-5
Olt GJ (*see* Soper et al). 1990;163:1204-9
Ordidge RJ (*see* Johnson et al). 1990;163:601-7
Ordorica SA (*see* Frieden et al). 1990;163:830-3
—— (*see* Hoskins et al). 1990;163:1944-7
Orme ML'E, Back DJ. Factors affecting the enterohepatic cir-

culation of oral contraceptive steroids. 1990;163:2146-52 (Symposium)
—— (*see* Back et al). 1990;163:2138-45 (Symposium)
Orr JW Jr, Orr PF, Barrett JM, Ellington JR Jr, Jennings RH Jr, Paredes KB, Patsner B, Taylor DL. Continuous or interrupted fascial closure: a prospective evaluation of No.1 Maxon suture in 402 gynecologic procedures. 1990;163:1485-9
Orr PF (*see* Orr et al). 1990;163:1485-9
Oskarsson T (*see* Dimitry et al). 1990;163:244-5 (Letter)
Ospitia JA (*see* Cabrera et al). 1990;163:2025 (Letter)
Osteen KG, Anderson TL, Hargrove JT, Hill GA, Gorstein F. Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro. 1990;163:479-84
Ostrea EM Jr (*see* Reyes and Ostrea). 1990;163:1376 (Letter reply)
Ouano L (*see* Wilson et al). 1990;163:195-202
Oury JF (*see* Cabrol et al). 1990;163:540-2
Owen J, Baker SL, Hauth JC, Goldenberg RL, Davis RO, Copper RL. Is indicated or spontaneous preterm delivery more advantageous for the fetus? 1990;163: 868-72
——, Goldenberg RL, Davis RO, Kirk KA, Copper RL. Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population. 1990;163:873-9
——, ——, ——, ——, ——. Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population (1990;163:873-9). 1990;163:2030 (Correction)
Owiny JR, Fitzpatrick RJ. Effect of intravaginal application of prostaglandin E_2 gel on the mechanical properties of the ovine cervix uteri at term. 1990;163:657-60

P

Palan PR (*see* Basu et al). 1990;163:1948-52
Paldi E (*see* Goldstein et al). 1990;163:1261-3
Palmer SM (*see* Dickinson et al). 1990;163:1831-5
—— (*see* Roth et al). 1990;163:1828-30
Palmer TV (*see* Roudebush et al). 1990;163:1670-3
Palomaki GE (*see* Haddow et al). 1990;163:1372 (Letter)
—— (*see* Johnson et al). 1990;163:9-11
Palow D (*see* Burkett et al). 1990;163:40-1
Palti Z. Treatment of cervical pregnancy with methotrexate. 1990;163:694 (Letter reply)
Pansky M (*see* Raziel et al). 1990;163:1182-5
Papenhausen P (*see* Carlan et al). 1990;163:490-3
Papoz L (*see* Simon et al). 1990;163:382-7 (Symposium)
Paredes KB (*see* Orr et al). 1990;163:1485-9
Parent A, Schiffrin EL, St-Louis J. Role of the endothelium in adrenergic responses of mesenteric artery rings of pregnant rats. 1990;163:229-34
Parisi VM (*see* Dickinson et al). 1990;163:1831-5
—— (*see* Thorp and Parisi). 1990;163:248-9 (Letter reply)
Parker WH, Berek JS. Management of selected cystic adnexal masses in postmenopausal women by operative laparoscopy: a pilot study. 1990;163:1574-7
Parks JS (*see* Adams et al). 1990;163:1388-92 (Symposium)
Parmley TH II (*see* Hardardottir et al). 1990;163:1925-31
Parsons MT (*see* Wilson et al). 1990;163:195-202
—— (*see* Wilson et al). 1990;163:1875-82
Pascoe-Mason J (*see* Droste et al). 1990;163:158-61
Pasic R, Wolfe WM. Laparoscopic diagnosis and treatment of interstitial ectopic pregnancy: a case report. 1990;163:587-8
Passaris G (*see* Brown et al). 1990;163:1154-6
Patalas E (*see* McCoshen et al). 1990;163:81-5
Patel J (*see* Harding et al). 1990;163:1566-8
Pater EA (*see* Low et al). 1990;163:1131-5
Patsner B. A comment on thumbtacks for hemorrhage control. 1990;163:1093 (Letter reply)
—— (*see* Orr et al). 1990;163:1485-9
Patterson RM (*see* Ridgway et al). 1990;163:879-82
—— (*see* Wright et al). 1990;163:748-50
—— (*see* Wright et al). 1990;163:889-92
Paul RH (*see* Kjos et al). 1990;163:898-903
—— (*see* Posner et al). 1990;163:813-8
—— (*see* Strong et al). 1990;163:1370-1 (Letter reply)
Paulick RP, Meyers RL, Rudolph CD, Rudolph AM. Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb. 1990;163:1357-63
Paulson RJ, Sauer MV, Lobo RA. Factors affecting embryo implantation after human in vitro fertilization: a hypothesis. 1990;163:2020-3
—— (*see* Sauer and Paulson). 1990;163:1421-4 (Clin. opinion)
Pawson IG (*see* Green et al). 1990;163:86-92
Payne FG (*see* Thorp et al). 1990;163:655-6
Payraudeau PH (*see* Basu et al). 1990;163:1948-52
Pearse WH. A conceptual thought. 1990;163:1098 (Letter)
Pechlaner C (*see* Kunz et al). 1990;163:417-20 (Symposium)
Peisner DB (*see* Guy et al). 1990;163:723-7
Peretz BA (*see* Goldstein et al). 1990;163:1261-3
Pergament E (*see* Ginsberg et al). 1990;163:1186-90
Perry J (*see* Green et al). 1990;163:86-92
Persutte WH (*see* Lenke et al). 1990;163:995-8
Peters MT (*see* Trimmer et al). 1990;163:1900-3
Petersen KR (*see* Jespersen et al). 1990;163:396-403 (Symposium)
—— (*see* Skouby et al). 1990;163:343-8 (Symposium)
Peterson HB (*see* Sanchez-Ramos et al). 1990;163:1081-8
Peterson IM, Braak B. Intrapartum uterine rupture and inflammatory bowel disease. 1990;163:1247-8
Petion AM (*see* Gouyon and Petion). 1990;163:1375 (Letter)
Petraglia F, Garuti GC, De Ramundo B, Angioni S, Genazzani AR, Bilezikjian LM. Mechanism of action of interleukin-1β in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells. 1990;163:1307-12
Petrella A (*see* Curiel et al). 1990;163:538-9
Petrie RH (*see* Shyken et al). 1990;163:802-7
Petrikovsky BM, Cohen M, Tancer ML. Fetal heart rate monitoring during cesarean section. 1990;163:253 (Letter reply)
Petty CN (*see* Mahony et al). 1990;163:1513-22
Pfeffer M (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Pham FK (*see* Samaan et al). 1990;163:1836-9
Philipson E (*see* Kalhan et al). 1990;163:250-1 (Letter reply)
Phillips A (*see* McGuire et al). 1990;163:2127-31 (Symposium)
Phillips CB (*see* Smith et al). 1990;163:845-7
Phillips OP (*see* Shulman et al). 1990;163:1606-9
Piacquadio KM, Brace RA, Cheung CY. Role of vasopressin in mediation of fetal cardiovascular responses to acute hypoxia. 1990;163:1294-300
—— (*see* Andres et al). 1990;163:935-8
—— (*see* Andres et al). 1990;163:1551-3
—— (*see* Moore and Piacquadio). 1990;163:264-5 (Letter reply)
Pieroni G (*see* Riveros et al). 1990;163:704-5 (Letter)
Pietrantoni M (*see* Carlan et al). 1990;163:490-3
Pinckert TL, Lebo RV, Golbus MS. Rapid determination of fetal sex. 1990;163:684 (Letter reply)
Pinto S (*see* Abbate et al). 1990;163:424-9 (Symposium)
Pipkin FB (*see* Baker et al). 1990;163:175-6

Pircon RA (*see* Towers et al). 1990;163:965-7
Pittaway DE. The use of serial CA 125 concentrations to monitor endometriosis in infertile women. 1990;163:1032-7
Piura B (*see* Glezerman et al). 1990;163:704 (Letter reply)
Plapp FV (*see* Thorp et al). 1990;163:607-9
Platt LD (*see* Carlson et al). 1990;163:1785-7
Plessinger MA, Woods JR Jr. Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes. 1990;163:1659-64
Plosker S (*see* Jaffe et al). 1990;163:1727-31 (Symposium)
Plouffe L Jr (*see* Odom et al). 1990;163:76-7
Pokras R (*see* Saftlas et al). 1990;163:460-5
Polk BF (*see* Harger et al). 1990;163:130-7
Pollack KL, Chestnut DH, Weiner CP, Thompson CS, DeBruyn CS. Magnesium sulfate and promethazine do not interact to cause hypotension in gravid ewes. 1990;163:787-94
Pollow K (*see* Juchem and Pollow). 1990;163:2171-83 (Symposium)
Pool R (*see* Ernest et al). 1990;163:978-80
Posner MD, Ballagh SA, Paul RH. The effect of amnioinfusion on uterine pressure and activity: a preliminary report. 1990;163:813-8
Powell C (*see* Smith et al). 1990;163:567-9
Pridjian G, Moawad AH. Pregnancy loss terminology. 1990;163:246 (Letter reply)
Pritchard JA (*see* Cunningham et al). 1990;163:453-9
Pritchard JR (*see* Choi and Pritchard). 1990;163:74-6
Provencher DM (*see* Rubin et al). 1990;163:69-73
Puistola U, Risteli L, Risteli J, Kauppila A. Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum. 1990;163:1276-81
Pulkkinen M, Jaakkola U-M. The complex nature of egg transport through the oviduct. 1990;163:687-8 (Letter reply)
Punch MR, Ansbacher R. Autogenic masculinization. 1990;163:114-6
Puppa AD, Torre LD, Favretti L. The local anesthetic dose is an important issue in evaluation of effects of epidural analgesia in nulliparous women. 1990;163:701-2 (Letter)

Q

Queenan J. Amniotic fluid midtrimester delta optical density measurements at 450 nm in Rh disease. 1990;163:711-2 (Letter reply)
Quinn DL (*see* Rodis et al). 1990;163:1168-71
Quirk JG (*see* Catanzarite and Quirk). 1990;163:1191-5
—— (*see* Clark and Quirk). 1990;163:1367-8 (Letter)
Quirk JG Jr (*see* Hardardottir et al). 1990;163:1925-31
Qvist I (*see* Bergink et al). 1990;163:2132-7 (Symposium)

R

Rajabi MR, Dean DD, Woessner JF Jr. Changes in active and latent collagenase in human placenta around the time of parturition. 1990;163:499-503
Rajendran V (*see* Al Kasab et al). 1990;163:37-40
Ramos E (*see* Devoe et al). 1990;163:1156-61
Rankin SE (*see* Jayo et al). 1990;163:614-8
Rao CV, Yussman MA, Hussa RO . Stability of human chorionic gonadotropin and its α-subunit. 1990;163:1098-9 (Letter)
Raskinet R (*see* David et al). 1990;163:420-3 (Symposium)
Rayburn WF (*see* Egarter et al). 1990;163:794-6
—— (*see* Smith et al). 1990;163:845-7
Raziel A, Golan A, Pansky M, Ron-El R, Bukovsky I, Caspi E. Ovarian pregnancy: a report of 20 cases in one institution. 1990;163:1182-5
Read JA (*see* Christian et al). 1990;163:848-55
Rech S (*see* Chatwani et al). 1990;163:240 (Letter)
Redaelli R (*see* Baudo et al). 1990;163:1274-5
Reece EA, Gabrielli S, Cullen MT, Zheng X-Z, Hobbins JC, Harris EN. Recurrent adverse pregnancy outcome and antiphospholipid antibodies. 1990;163:162-9 (Curr. dev.)
Reed KL (*see* Indik and Reed). 1990;163:1792-6
Refn H, Kjæaer A, Lebech A-M, Borggaard B, Schierup L, Bremmelgaard A. Metabolic changes during treatment with two different progestogens. 1990;163:374-7 (Symposium)
Reichman BS (*see* Rubin et al). 1990;163:69-73
Rein MS (*see* Friedman et al). 1990;163:1114-9 (Clin. opinion)
Reiter AA (*see* Smith et al). 1990;163:567-9
Reiter RC (*see* Gambone et al). 1990;163:545-50
Rekers H (*see* Kloosterboer and Rekers). 1990;163:370-3 (Symposium)
Repke JT (*see* Villar and Repke). 1990;163:1124-31
Resano FG (*see* Burke et al). 1990;163:17-8
Resnick MB, Ariet M. Mortality rates ignore population segment. 1990;163:269-70 (Letter reply)
Resnik R (*see* Andres et al). 1990;163:935-8
—— (*see* Andres et al). 1990;163:1551-3
—— (*see* Gilbert et al). 1990;163:998-9
Reuwen A. Prediction of residual trophoblastic tissue in first-trimester abortions: the role of transvaginal Doppler ultrasonography. 1990;163:2024-5 (Letter)
Reyes MP, Ostrea EM Jr. Toxicity of vancomycin given during pregnancy. 1990;163:1376 (Letter reply)
Ribbert LSM, Snijders RJM, Nicolaides KH, Visser GHA. Relationship of fetal biophysical profile and blood gas values at cordocentesis in severely growth-retarded fetuses. 1990;163:569-71
Richards DS (*see* Johnson and Richards). 1990;163:1364 (Letter reply)
Richart RM (*see* Ferenczy et al). 1990;163:1271-4
Richter R (*see* McGregor et al). 1990;163:1465-73
—— (*see* McGregor et al). 1990;163:1580-91
Ricker DD (*see* Roudebush et al). 1990;163:1670-3
Ridgway LE (*see* Wright and Ridgway). 1990;163:1788-91
Ridgway LE III, Muise K, Wright JW, Patterson RM, Newton ER. A prospective randomized comparison of oral terbutaline and magnesium oxide for the maintenance of tocolysis. 1990;163:879-82
—— (*see* Wright et al). 1990;163:748-50
—— (*see* Wright et al). 1990;163:889-92
Riley SC (*see* Cheung et al). 1990;163:1445-9
Risteli J (*see* Puistola et al). 1990;163:1276-81
Risteli L (*see* Puistola et al). 1990;163:1276-81
Ritchie JWK (*see* Morrow et al). 1990;163:1313-20
Riveros DA, Grand BE, Blanco AN, Pieroni G, Lazzari M, Voto LS. Laboratory identification of the lupus anticoagulant in normal pregnancy and pregnancy-induced hypertension. 1990;163:704-5 (Letter)
Rizk B. Embryo migration responsible for ectopic pregnancies. 1990;163:1369 (Letter)
Rizzo G, Nicolaides KH, Arduini D, Campbell S. Effects of intravascular fetal blood transfusion on fetal intracardiac Doppler velocity waveforms. 1990;163:1231-8
Rizzo T, Freinkel N, Metzger BE, Hatcher R, Burns WJ, Barglow P. Correlations between antepartum maternal metabolism and newborn behavior. 1990;163:1458-64
Roberts A (*see* Romero et al). 1990;163:823-5
Roberts AB, Mitchell JM. Direct ultrasonographic measure-

ment of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes. 1990;163:1560-6
Roberts JM, Taylor RN. Preeclampsia—An endothelial cell disorder plus "something else"? 1990;163:1366 (Letter reply)
—— (*see* Taylor et al). 1990;163:1839-44
Roberts WS (*see* Cavanagh et al). 1990;163:1007-15
—— (*see* LaPolla et al). 1990;163:1055-60
Robertson PA (*see* Combs et al). 1990;163:100-4
Robertson WG (*see* Nordin et al). 1990;163:140-5
Robinsin C (*see* Meizner et al). 1990;163:128-9
Robinson GE (*see* Stewart et al). 1990;163:1196-9
Rock JA (*see* Xu et al). 1990;163:1493-7
Rodeck CH (*see* Nicolini et al). 1990;163:1104 (Letter reply)
—— (*see* Nicolini et al). 1990;163:1144-6
Rodis JF, Quinn DL, Gary GW Jr, Anderson LJ, Rosengren S, Cartter ML, Campbell WA, Vintzileos AM. Management and outcomes of pregnancies complicated by human B19 parvovirus infection: a prospective study. 1990;163:1168-71
Rodriquez J (*see* Moretti et al). 1990;163:833-8
Rodway MR, Yuen BH, Leung PCK. Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast. 1990;163:1546-51
Roe DA, Little BB, Bawdon RE, Gilstrap LC III. Metabolism of cocaine by human placentas: implications for fetal exposure. 1990;163:715-8
—— (*see* Harstad et al). 1990;163:912-6
Rogo K (*see* Stendahl and Rogo). 1990;163:685-6 (Letter)
Romero K (*see* Macri et al). 1990;163:1248-53
Romero R, Hsu YC, Athanassiadis AP, Hagay Z, Avila C, Nores J, Roberts A, Mazor M, Hobbins JC. Preterm delivery: a risk factor for retained placenta. 1990;163:823-5
——, Jimenez C, Lohda AK, Nores J, Hanaoka S, Avila C, Callahan R, Mazor M, Hobbins JC, Diamond MP. Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor. 1990;163:968-74
——, Shamma F, Avila C, Jimenez C, Callahan R, Nores J, Mazor M, Brekus CA, Hobbins JC. Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in twin gestations with preterm labor. 1990;163:757-61
Romney SL (*see* Basu et al). 1990;163:1948-52
Ron-El R (*see* Raziel et al). 1990;163:1182-5
Rosati D (*see* Abbate et al). 1990;163:424-9 (Symposium)
Rose JC (*see* Cuthrell et al). 1990;163:170-4
—— (*see* Hatjis et al). 1990;163:45-50
Rosenfeld CR (*see* Brown et al). 1990;163:1853-61
Rosenfield A (*see* Chavkin and Rosenfield). 1990;163:450-2 (Clin. opinion)
Rosengren S (*see* Rodis et al). 1990;163:1168-71
Rosenn B (*see* Stamler et al). 1990;163:1217-21
Ross GS (*see* Lipper et al). 1990;163:146-50
Ross MG. Dehydration-induced oligohydramnios. 1990;163:1091-2 (Letter)
—— (*see* Cardin et al). 1990;163:1345-9
Rossi E (*see* Kalhan et al). 1990;163:250-1 (Letter reply)
Rostagno C (*see* Abbate et al). 1990;163:424-9 (Symposium)
Rote NS, Dostal-Johnson D, Branch DW. Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin. 1990;163:575-84
Roth JB, Thorp JA, Palmer SM, Brath PC, Walsh SW, Crandell SS. Response of placental vasculature to high glucose levels in the isolated human placental cotyledon. 1990;163:1828-30
Roudebush WE, Minhas BS, Ricker DD, Palmer TV, Dodson MG. Platelet activating factor enhances in vitro fertilization of rabbit oocytes. 1990;163:1670-3
Roussis P (*see* Jeanty et al). 1990;163:50-1
Rovati V, Faleschini E, Vercellini P, Nervetti G, Tagliabue G, Benzi G. Endometrioma of the liver. 1990;163:1490-2
Rozas L (*see* Katz et al). 1990;163:1142-3
Rubin SC, Finstad CL, Hoskins WJ, Saigo PE, Provencher DM, Federici MG, Hakes TB, Markman M, Reichman BS, Lloyd KO, Lewis JL Jr. Expression of P-glycoprotein in epithelial ovarian cancer: evaluation as a marker of multidrug resistance. 1990;163:69-73
Rubinstein A (*see* Minkoff et al). 1990;163:1598-604
Rubio E (*see* Martinez-Mir et al). 1990;163:189-95
Rudolph AM (*see* Paulick et al). 1990;163:1357-63
Rudolph CD (*see* Paulick et al). 1990;163:1357-63
Ruffolo E (*see* LaPolla et al). 1990;163:1055-60
Rushant S (*see* Mooney et al). 1990;163:30-6
Ryan G. Phenytoin and magnesium sulfate in eclampsia. 1990;163:1090-1 (Letter reply)
Rydhström H. Prognosis for twins with birth weight 1500 gm: the impact of cesarean section in relation to fetal presentation. 1990;163:528-33

S

Sabag T (*see* Al Kasab et al). 1990;163:37-40
Sabbagha RE (*see* Keller et al). 1990;163:893-7
Sadiq F (*see* Devaskar et al). 1990;163:1350-6
Sadler TW (*see* Smoak and Sadler). 1990;163:619-24
Sadovsky Y, Amon E. Amnioinfusion and prevention of meconium aspiration syndrome. 1990;163:259 (Letter reply)
Saftlas AF, Olson DR, Franks AL, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986. 1990;163:460-5
Saigo PE (*see* Rubin et al). 1990;163:69-73
St-Louis J (*see* Parent et al). 1990;163:229-34
Saint-Paul M (*see* Simon et al). 1990;163:382-7 (Symposium)
Saji F (*see* Nobunaga et al). 1990;163:634-8
Sakata M, Terakawa N, Mizutani T, Tanizawa O, Matsumoto K, Terada N, Sudo K. Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis. 1990;163:1679-84
Salafia CM, Vintzileos AM. Why all placentas should be examined by a pathologist in 1990. 1990;163:1282-93 (Curr. dev.)
—— (*see* Cullen et al). 1990;163:466-76
Saller DN Jr, Keene CL, Sun C-CJ, Schwartz S. The association of single umbilical artery with cytogenetically abnormal pregnancies. 1990;163:922-5
Salwen M (*see* Minkoff et al). 1990;163:521-6
Samaan NA, Schultz PN, Pham FK. Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns. 1990;163:1836-9
Samsioe G, Mattsson L-[oc]A. Some aspects of the relationship between oral contraceptives, lipid abnormalities, and cardiovascular disease. 1990;163:354-8 (Symposium)
Samuels SJ (*see* Gillogley et al). 1990;163:1535-42
Sanchez-Ramos L, Kaunitz AM, Peterson HB, Martinez-Schnell B, Thompson RJ. Reducing cesarean sections at a teaching hospital. 1990;163:1081-8

—— (*see* Johnston et al). 1990;163:743-7
Sanders MM (*see* Hardardottir et al). 1990;163:1925-31
Santen RJ (*see* Wild et al). 1990;163:1998-2005
Santoro N. Efficacy and safety of intravenous pulsatile gonadotropin-releasing hormone: Lutrepulse for injection. 1990;163:1759-64 (Symposium)
Sanyal MK (*see* Grifo et al). 1990;163:2013-9
Sarno AP Jr (*see* Letterie et al). 1990;163:1100 (Letter)
Sasaki A. Corticotropin-releasing hormone in amniotic fluid. 1990;163:1102-3 (Letter reply)
Satah S (*see* Horimoto et al). 1990;163:1480-4
Sauer MV, Paulson RJ. Human oocyte and preembryo donation: an evolving method for the treatment of infertility. 1990;163:1421-4 (Clin. opinion)
—— (*see* Paulson et al). 1990;163:2020-3
Saving KL, McLaren RA, Miller TC, O'Connor M. Responsibility expands to extended family members. 1990;163:1369-70 (Letter)
Savvas M (*see* Studd et al). 1990;163:1474-9
Sawyer W (*see* Al Kasab et al). 1990;163:37-40
Schaffer AV (*see* Cardin et al). 1990;163:1345-9
Schatzki SC (*see* Korn et al). 1990;163:596-8
Scheen AJ (*see* Jandrain et al). 1990;163:378-81 (Symposium)
Schiedermayer DL (*see* La Puma et al). 1990;163:680 (Letter reply)
Schierup L (*see* Refn et al). 1990;163:374-7 (Symposium)
Schiff E, Moran O, Ben-Baruch G, Menczer J. Measures of test performance and predictive values. 1990;163:1375 (Letter reply)
Schiffman JS (*see* Digre et al). 1990;163:1848-52
Schiffrin EL (*see* Parent et al). 1990;163:229-34
Schlafer D (*see* Copel et al). 1990;163:751-6
Schlaff W (*see* Xu et al). 1990;163:1493-7
Schlesselman JJ. Oral contraceptives and breast cancer. 1990;163:1379-87 (Symposium)
Schnatterly PT (*see* Hogge et al). 1990;163:698 (Letter reply)
Schneider D (*see* Langer et al). 1990;163:1139-41
Schoonmaker J (*see* McGregor et al). 1990;163:1580-91
Schriock ED. Practical aspects of pulsatile gonadotropin-releasing hormone administration. 1990;163:1765-70 (Symposium)
Schultz PN (*see* Samaan et al). 1990;163:1836-9
Schumacher LB (*see* Green et al). 1990;163:86-92
Schütt B (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Schutz T (*see* Ferguson et al). 1990;163:105-11
Schwartz S (*see* Saller et al). 1990;163:922-5
Sciarra JJ (*see* Valle and Sciarra). 1990;163:1106-7 (Letter reply)
Scott JR (*see* Branch and Scott). 1990;163:245-6 (Letter)
Scott RT (*see* Hankins et al). 1990;163:728-32
Scully RE (*see* Korn et al). 1990;163:596-8
Sealey JE (*see* August et al). 1990;163:1612-21
Sedlis A (*see* Hoegsberg et al). 1990;163:1135-9
Seeds JW (*see* Watson et al). 1990;163:1456-7
Seeley DG (*see* Cauley et al). 1990;163:1438-44
Seifer DB, Jones EE. Parameters of hypothalamic aging. 1990;163:1715 (Letter)
Seifert W (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Senan C (*see* Simon et al). 1990;163:382-7 (Symposium)
Seo K (*see* McGregor et al). 1990;163:1580-91
Sert MB. Role of hormones in cervical ripening. 1990;163:705-7 (Letter)
Sever JL. The importance of considering clinical usefulness in the evaluation of medical laboratory tests. 1990;163:678-9 (Letter)
Shah DM, Chaffin D. Twin transfusion syndrome. 1990;163:1377 (Letter reply)
—— (*see* Jeanty et al). 1990;163:50-1
Shahed M (*see* Al Kasab et al). 1990;163:37-40
Shamma F (*see* Romero et al). 1990;163:757-61
Shanklin DR, Sibai BM. Ultrastructural aspects of preeclampsia. II. Mitochondrial changes. 1990;163:943-53
—— (*see* Barton et al). 1990;163:534-8
Shaver D (*see* Moretti et al). 1990;163:833-8
Sheckter CB (*see* Matsumoto et al). 1990;163:1743-51 (Symposium)
Shepard TH (*see* Droste et al). 1990;163:158-61
Shepherd JH (*see* van Dam et al). 1990;163:698-9 (Letter)
Sherer DM. Treatment of cervical pregnancy with methotrexate. 1990;163:693-4 (Letter)
——, Abramowicz JS. Possible therapeutic applications of cordocentesis. 1990;163:1366-7 (Letter)
——, Cullen JBH, Thompson HO, Woods JR Jr. Dehydration-induced oligohydramnios. 1990;163:1092 (Letter reply)
—— (*see* Sites et al). 1990;163:1630-1
Sherman ML (*see* Cox et al). 1990;163:767-72
Shettles LB. How sperm commence movement and their isolation for in vitro fertilization and sex selection. 1990;163:270 (Letter)
——. Tubal embryo successfully transferred in utero. 1990;163:2026-7 (Letter)
Shimonovitz S (*see* Ben-Chetrit et al). 1990;163:1161-3
Shiono PH (*see* Klebanoff et al). 1990;163:1450-6
Shirakawa K (*see* Izumi et al). 1990;163:638-47
Shirey RS (*see* Witter et al). 1990;163:784-6
Shively CA (*see* Adams et al). 1990;163:1388-92 (Symposium)
Sholl JS (*see* Silver et al). 1990;163:917-22
Shoupe D, Mishell DR Jr, Fossum G, Bopp B, Spitz IM, Lobo RA. Antiprogestin treatment decreases midluteal luteinizing hormone pulse amplitude and primarily exerts a pituitary inhibition. 1990;163:1982-5
—— (*see* Kjos et al). 1990;163:1822-7
Shulman LP, Tharapel AT, Meyers CM, Phillips OP, Simpson JL, Elias S. Direct analysis of uncultured cytotrophoblastic cells from second-and third-trimester placentas: an accurate and rapid method for detection of fetal chromosome abnormalities. 1990;163:1606-9
Shultz KL (*see* Tennent et al). 1990;163:625-34
Shyken JM, Smeltzer JS, Baxi LV, Blakemore KJ, Ambrose SE, Petrie RH. A comparison of the effect of epidural, general, and no anesthesia on funic acid-base values by stage of labor and type of delivery. 1990;163:802-7
Sibai BM. Eclampsia. VI. Maternal-perinatal outcome in 254 consecutive cases. 1990;163:1049-55
——. HELLP does not always mean immediate HELP! 1990;163:1089 (Letter reply)
——. Magnesium sulfate is not an ideal anticonvulsant. 1990;163:1715 (Letter reply)
——. Magnesium supplementation in pregnancy. 1990;163:241 (Letter reply)
——, Akl S, Fairlie F, Moretti M. A protocol for managing severe preeclampsia in the second trimester. 1990;163:733-8
—— (*see* Barton et al). 1990;163:534-8
—— (*see* Feldman et al). 1990;163:855-8
—— (*see* Mabie et al). 1990;163:1861-7
—— (*see* Moretti et al). 1990;163:833-8
—— (*see* Moretti et al). 1990;163:1844-8
—— (*see* Shanklin and Sibai). 1990;163:943-53
Sicuranza B (*see* Bardeguez et al). 1990;163:42-5

Siddiqi T (*see* Stamler et al). 1990;163:1217-21
Silver HM, Gibbs RS, Gray BM, Dillon HC. Risk factors for perinatal group B streptococcal disease after amniotic fluid colonization. 1990;163:19-25
Silver RK, MacGregor SN, Sholl JS, Hobart ED, Waldee JK. An evaluation of the chorionic villus sampling learning curve. 1990;163:917-22
——, Turbov JM, Beaird JA, Golbus J. Soluble factors produced by isolated first-trimester chorionic villi directly inhibit proliferation of T cells. 1990;163:1914-9
Simke JP (*see* Cardin et al). 1990;163:1345-9
Simon D, Senan C, Garnier P, Saint-Paul M, Garat E, Thibult N, Papoz L. Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom Study. 1990;163:382-7 (Symposium)
Simpson JL (*see* Shulman et al). 1990;163:1606-9
Sindwani V, Verma RS. Cytogenetic uncertainties surrounding the fragile X in Martin-Bell syndrome. 1990;163:1713-4 (Letter)
Singh R (*see* Nestler et al). 1990;163:1243-6
Sites CK, Sherer DM, Gandell DL, Woods JR Jr. Extensive vulvar and vaginal varicella necessitating abdominal delivery. 1990;163:1630-1
Sjöblom P (*see* Lindblom et al). 1990;163:260-1 (Letter reply)
—— (*see* Lindblom et al). 1990;163:694-5 (Letter reply)
Skajaa K, Svane D, Andersson K-E, Forman A. Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacetal arteries in term pregnancy. 1990;163:1323-33.
Skoll A (*see* Feldman et al). 1990;163:855-8
Skouby SO, Andersen O, Petersen KR, Mølsted-Pedersen L, Kühl C. Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level. 1990;163:343-8 (Symposium)
—— (*see* Jespersen et al). 1990;163:273 (Symposium)
—— (*see* Jespersen et al). 1990;163:396-403 (Symposium)
Slade S (*see* Mills et al). 1990;163:12-7
Smeltzer JS (*see* Kivikoski et al). 1990;163:123-8
—— (*see* Shyken et al). 1990;163:802-7
Smith CV, Rayburn WF, Connor RE, Fredstrom GR, Phillips CB. Double-blind comparison of intravaginal prostaglandin E_2 gel and "chip" for preinduction cervical ripening. 1990;163:845-7
Smith ER (*see* Chuong et al). 1990;163:1591-5
Smith LG Jr, Carpenter RJ Jr, Gonsoulin W, Mari G, Reiter AA, Greenberg F, Powell C. Prenatal diagnosis of a chest wall mass with ultrasonography and Doppler velocimetry: a case report. 1990;163:567-9
—— Kirshon B, Cotton DB. Indomethacin treatment for polyhydramnios and subsequent infantile nephrogenic diabetes insipidus. 1990;163:98-9
Smoak IW, Sadler TW. Embryopathic effects of short-term exposure to hypoglycemia in mouse embryos in vitro. 1990;163:619-24
Smythe AR II, McFarland KF, Yousufuddin M, Meredith WD. Multiple endocrine adenomatosis type I in pregnancy 1990;163:1037-9
Snijders RJM (*see* Ribbert et al). 1990;163:569-71
Snyder JR (*see* Goldstein et al). 1990;163:119-23
Snyder S, Cardwell MS. Combination therapy has potential complications. 1990;163:244 (Letter reply)
Socol ML (*see* Utter et al). 1990;163:882-6
—— (*see* Utter et al). 1990;163:1781-4
Soffici A (*see* Tabor and Soffici). 1990;163:1364-5 (Letter)
Soisson AP (*see* Soper et al). 1990;163:1204-9
Sokol RJ. Use of the T-ACE questions to detect risk-drinking. 1990;163:684-5 (Letter reply)
Sölder E (*see* Kunz et al). 1990;163:417-20 (Symposium)
Sollenberger MJ, Carlsen EC, Booth RA Jr, Johnson ML, Veldhuis JD, Evans WS. Nature of gonadotropin-releasing hormone self-priming of luteinizing hormone secretion during the normal menstrual cycle. 1990;163:1529-34
Sonek J (*see* Kniss et al). 1990;163:1883-90
Songster C (*see* LaPolla et al). 1990;163:1055-60
Sonta JA (*see* Cederna et al). 1990;163:138-9
Soper DE, Bump RC, Hurt WG. Bacterial vaginosis and trichomoniasis vaginitis are risk factors for cuff cellulitis after abdominal hysterectomy. 1990;163:1016-23
Soper JT, Berchuck A, Olt GJ, Soisson AP, Clarke-Pearson DL, Bast RC Jr. Preoperative evaluation of serum CA 125, TAG 72, and CA 15-3 in patients with endometrial carcinoma. 1990;163:1204-9
—— (*see* Hunter et al). 1990;163:1164-7
Sorrells TL (*see* McColgin et al). 1990;163:1503-5
Sowers JR, Zemel MB. Intracellular sodium levels in pregnant women with hypertension. 1990;163:262 (Letter reply)
Spätling L, Fallenstein F. Magnesium supplementation in pregnancy. 1990;163:240-1 (Letter)
Speizer FE (*see* Stampfer et al). 1990;163:285-91 (Symposium)
Spielman FJ (*see* Thorp et al). 1990;163:655-6
Spinelli G (*see* Curiel et al). 1990;163:538-9
Spitz B (*see* Brown et al). 1990;163:1853-61
Spitz IM (*see* Shoupe et al). 1990;163:1982-5
Stabler M (*see* Kjos et al). 1990;163:898-903
Stale H, Gennser G, Mar[brev]sál K. Blood flow velocity and pulsatile diameter changes in the fetal descending aorta: a longitudinal study. 1990;163:26-9
Stamler EF, Cruz ML, Mimouni F, Rosenn B, Siddiqi T, Khoury J, Miodovnik M. High infectious morbidity in pregnant women with insulin-dependent diabetes: an understated complication. 1990;163:1217-21
Stampfer MJ, Willett WC, Colditz GA, Speizer FE, Hennekens CH. Past use of oral contraceptives and cardiovascular disease: a meta-analysis in the context of the Nurses' Health Study. 1990;163:285-91 (Symposium)
Stanczyk FZ (*see* Cassidenti et al). 1990;163:1953-60
Stark CF, Gibbs RS, Freedman WL. Comparison of umbilical artery pH and 5-minute Apgar score in the low-birth-weight and very-low-birth-weight infant. 1990;163:818-23
Steele B (*see* Burkett et al). 1990;163:40-1
Stehling MK (*see* Johnson et al). 1990;163:501-7
Steiger RM, Nageotte MP. Effect of uterine contractility and maternal hypotension on prolonged decelerations after bupivacaine epidural anesthesia. 1990;163:308-12
Steinberg B (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Stendahl U, Rogo K. Cervical cancer: Role for progesterone during pregnancy and contraception? 1990;163:685-6 (Letter)
Stephens JD. Accuracy of apical four-chamber view as screen for congenital heart disease. 1990;163:249-50 (Letter)
——. Rapid determination of fetal sex. 1990;163:683-4 (Letter)
——. Should amniocenteses always accompany unexplained high maternal serum α-fetoprotein levels? Who decides: public agency, patient, or private physician? 1990;163:697-8 (Letter)
——. Ultrasonography's role in detection of intrauterine synechiae. 1990;163:1106 (Letter)
Stephens P (*see* Carlan et al). 1990;163:490-3
Stephenson S (*see* Thorp et al). 1990;163:607-9

Stern JL, Major C, Van Le L. Preventing cervical conization by achieving satisfactory colposcopy with hygroscopic cervical dilators. 1990;163:176-7
Stern MP (*see* Berkus et al). 1990;163:1812-7
—— (*see* Berkus et al). 1990;163:1818-21
Sternlicht D (*see* O'Brien et al). 1990;163:1796-8
Stevens R (*see* Minkoff et al). 1990;163:521-6
Stevenson DK (*see* Ferguson et al). 1990;163:105-11
Stewart DE, Robinson GE, Goldbloom DS, Wright C. Infertility and eating disorders. 1990;163:1196-9
Stewart PA (*see* van Eyck et al). 1990;163:1239-42
Stiller RJ. Vaginal probe ultrasonography and ectopic pregnancy. 1990;163:1099-100 (Letter reply)
Stratton P (*see* Minkoff et al). 1990;163:1598-604
Strauss AW (*see* Long et al). 1990;163:1976-81
Strickland DM, Whitted WA, Wians FH Jr. Screening infertile women for subclinical hypothyroidism. 1990;163:262-3 (Letter)
——, Yeomans ER, Hankins GDV. Cost-effectiveness of intrapartum screening and treatment for maternal group B streptococci colonization. 1990;163:4-8 (Clin. opinion)
Strom S (*see* Kauma et al). 1990;163:1430-7
Strong TH Jr, Hetzler G, Paul RH. Does prophylactic amnioinfusion make a difference? 1990;163:1370-1 (Letter reply)
Stuart CA (*see* Nagamani and Stuart). 1990;163:1992-7
Studd J, Savvas M, Waston N, Garnett T, Fogelman I, Cooper D. The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants. 1990;163:1474-9
Su H-C (*see* Kniss et al). 1990;163:1883-90
Sudo K (*see* Sakata et al). 1990;163:1679-84
Sudo N (*see* Takahashi et al). 1990;163:112-3
Sun C-CJ (*see* Saller et al). 1990;163:922-5
Sundberg JP (*see* Tennent et al). 1990;163:625-34
Sunderji SG (*see* Macri et al). 1990;163:1373-4 (Letter reply)
Suzuki DK, Miller G. Maternal exercise and early pregnancy outcome. 1990;163:1103 (Letter)
Svane D (*see* Skajaa et al). 1990;163:1323-33
Swain M (*see* Hatjis et al). 1990;163:45-50
Swindells HE (*see* Mooney et al). 1990;163:30-6
Sworniowski T (*see* Cederna et al). 1990;163:138-9
Symonds EM (*see* Baker et al). 1990;163:175-6
—— (*see* Johnson et al). 1990;163:601-7
Szekely Z (*see* Cabrera et al). 1990;163:2025 (Letter)
Szekeres-Bartho J, Chaouat G, Kinsky R. A progesterone-induced blocking factor corrects high resorption rates in mice treated with antiprogesterone. 1990;163:1320-1
Szeto HH, Hinman DJ. Differential sensitivities of fetal muscle groups to *d*-tubocurarine. 1990;163:202-9

T

Tabarelli M (*see* Kunz et al). 1990;163:417-20 (Symposium)
Tabor BL, Ikegami M, Jobe AH, Yamada T, Oetomo SB. Dose response of thyrotropin-releasing hormone on pulmonary maturation in corticosteroid-treated preterm rabbits. 1990;163:669-76
——, Soffici A. Cocaine effects on fetal behavioral state. 1990;163:1364-5 (Letter)
Tagliabue G (*see* Rovati et al). 1990;163:1490-2
Tai H-H (*see* Cheung et al). 1990;163:1445-9
Takahashi H, Kanazawa K, Ikarashi T, Sudo N, Tanaka K. Discrepancy in the diagnoses of hydatidiform mole by macroscopic and microscopic findings and the deoxyribonucleic acid fingerprint method. 1990;163:112-3
Takemura M (*see* Nobunaga et al). 1990;163:634-8
Tamura RK (*see* Keller et al). 1990;163:893-7
—— (*see* Martin et al). 1990;163:1209-16
—— (*see* Utter et al). 1990;163:882-6
Tanaka K (*see* Takahashi et al). 1990;163:112-3
Tanaka S (*see* Koga et al). 1990;163:1543-5
Tanaka T (*see* Koga et al). 1990;163:1543-5
Tancer ML (*see* Goldstein et al). 1990;163:589-91
—— (*see* Petrikovsky et al). 1990;163:253 (Letter reply)
Tanizawa O (*see* Nobunaga et al). 1990;163:634-8
—— (*see* Sakata et al). 1990;163:1679-84
Tannirandorn Y (*see* Nicolini et al). 1990;163:1144-6
Täuber U, Kuhnz W, Hümpel M. Pharmacokinetics of gestodene and ethinyl estradiol after oral administration of a monophasic contraceptive. 1990;163:1414-20 (Symposium)
—— (*see* Hümpel et al). 1990;163:329-33 (Symposium)
Taylor DL (*see* Orr et al). 1990;163:1485-9
Taylor PV, Campbell JM. Association between autoantibodies and unexplained infertility unproved . 1990;163:243 (Letter reply)
Taylor RN, Heilbron DC, Roberts JM. Growth factor activity in the blood of women in whom preeclampsia develops is elevated from early pregnancy. 1990;163:1839-44
—— (*see* Kratzer and Taylor). 1990;163:1497-502
—— (*see* Roberts and Taylor). 1990;163:1366 (Letter reply)
Teichmann AT. Influence of oral contraceptives on drug therapy. 1990;163:2208-13 (Symposium)
Tennent BJ, Shultz KL, Sundberg JP, Beamer WG. Ovarian granulosa cell tumorigenesis in SWR-derived F1 hybrid mice: preneoplastic follicular abnormality and malignant disease progression. 1990;163:625-34
Tepedino M (*see* Bardeguez et al). 1990;163:42-5
Terada N (*see* Sakata et al). 1990;163:1679-84
Terakawa N (*see* Sakata et al). 1990;163:1679-84
Tharapel AT (*see* Shulman et al). 1990;163:1606-9
Thiagarajah S (*see* Hogge et al). 1990;163:698 (Letter reply)
—— (*see* Hogge et al). 1990;163:1061-5
Thibult N (*see* Simon et al). 1990;163:382-7 (Symposium)
Thijn CJP (*see* van Loon et al). 1990;163:1256-60
Tho SPT, Behzadian A, Byrd JR, McDonough PG. Correlation of the testicular determinant factor sequence zinc finger Y with varying gonadal phenotypes in a series of 13 subjects with gonadal dysgenesis because of Y aneuploidy. 1990;163:1968-75
Thomas K, Vankrieken L. Inhibition of ovulation by low-dose monophasic contraceptive containing gestodene. 1990;163:1404-9 (Symposium)
Thomason JL (*see* Livengood et al). 1990;163:515-20
Thompson CS (*see* Pollack et al). 1990;163:787-94
Thompson HO, Casaceli C, Woods JR Jr. Ultrasonographic fetal weight estimation by an integrated computer-assisted system: Can each laboratory improve its accuracy? 1990;163:986-95
—— (*see* Sherer et al). 1990;163:1092 (Letter reply)
Thompson RJ (*see* Sanchez-Ramos et al). 1990;163:1081-8
Thorneycroft IH. Oral contraceptives and myocardial infarction. 1990;163:1393-7 (Symposium)
Thorogood M, Vessey MP. An epidemiologic survey of cardiovascular disease in women taking oral contraceptives. 1990;163:274-81 (Symposium)
Thorp JA. The local anesthetic dose is an important issue in evaluation of effects of epidural analgesia in nulliparous women. 1990;163:702-3 (Letter reply)

——. What is birth asphyxia? 1990;163:1368 (Letter reply)
——, Parisi VM. Epidural analgesia and cesarean section for dystocia. 1990;163:248-9 (Letter reply)
——, Plapp FV, Cohen GR, Yeast JD, O'Kell RT, Stephenson S. Hyperkalemia secondary to irradiation of packed red blood cells: possible effects with intravascular fetal transfusion. 1990;163:607-9
—— (*see* Roth et al). 1990;163:1828-30
Thorp JM Jr, Bowes WA Jr. Vaginal birth after abdominal delivery. 1990;163:1101-2 (Letter)
——, Spielman FJ, Valea FA, Payne FG, Mueller RA, Cefalo RC. Nifedipine enhances the cardiac toxicity of magnesium sulfate in the isolated perfused Sprague-Dawley rat heart. 1990;163:655-6
—— (*see* Katz et al). 1990;163:1142-3
—— (*see* Watson et al). 1990;163:1456-7
Thoulon JM (*see* Cabrol et al). 1990;163:540-2
Tibaldi JM, Lorber DL, Nerenberg A. Diabetic ketoacidosis and insulin resistance with subcutaneous terbutaline infusion: a case report. 1990;163:509-10
Tikkanen MJ. Role of plasma lipoproteins in the pathogenesis of atherosclerotic disease, with special reference to sex hormone effects. 1990;163:296-304 (Symposium)
Timor-Tritsch IE (*see* Guy et al). 1990;163:723-7
Todd JK (*see* McGregor et al). 1990;163:1465-73
—— (*see* McGregor et al). 1990;163:1580-91
Todd MW (*see* Johnston et al). 1990;163:743-7
Tokugawa Y (*see* Nobunaga et al). 1990;163:634-8
Toledo AA (*see* Wiskind et al). 1990;163:1674-8
Tolman EL (*see* McGuire et al). 1990;163:2127-31 (Symposium)
Tonetta SA (*see* Levin et al). 1990;163:1932-8
Torre LD (*see* Puppa et al). 1990;163:701-2 (Letter)
Torres C (*see* O'Brien et al). 1990;163:1796-8
Toumala RE (*see* Harger et al). 1990;163:130-7
Towers CV, Garite TJ, Friedman WW, Pircon RA, Nageotte MP. Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients. 1990;163:965-7
—— (*see* Lewis et al). 1990;163:773-5
Treisser A (*see* Cabrol et al). 1990;163:540-2
Trimmer KJ, Leveno KJ, Peters MT, Kelly MA. Observations on the cause of oligohydramnios in prolonged pregnancy. 1990;163:1900-3
Trudinger B (*see* Giles et al). 1990;163:1713 (Letter)
Tucci M, Bard H. The associated anomalies that determine prognosis in congenital omphaloceles. 1990;163:1646-9
Tulandi T, Collins JA, Burrows E, Jarrell JF, McInnes RA, Wrixson W, Simpson CW. Treatment-dependent and treatment-independent pregnancy among women with periadnexal adhesions (1990;162:354-7). 1990;163:271 (Correction)
Tur-Kaspa I. Hyperbaric oxygen therapy for air embolism complicating operative hysteroscopy. 1990;163:680-1 (Letter)
Turbov JM (*see* Silver et al). 1990;163:1914-9
Turner T (*see* Kauma et al). 1990;163:1430-7

U

Uchiumi Y (*see* Izumi et al). 1990;163:638-47
Uguru V (*see* Lenox et al). 1990;163:1173-9
Uhlmann WR (*see* Johnson et al). 1990;163:1505-10
Ulmann A (*see* Cabrol et al). 1990;163:540-2
Ulmann D (*see* Cabrol et al). 1990;163:540-2
Urig MA, Clewell WH, Elliott JP. Twin-twin transfusion syndrome. 1990;163:1522-6
Urman B (*see* Durukan et al). 1990;163:594-5
Utter GO, Dooley SL, Tamura RK, Socol ML. Awaiting cervical change for the diagnosis of preterm labor does not compromise the efficacy of ritodrine tocolysis. 1990;163:882-6
——, Socol ML, Dooley SL, MacGregor SN, Millard DD. Is intrauterine transfusion associated with diminished fetal growth? 1990;163:1781-4

V

Vaag A (*see* Beck-Nielsen et al). 1990;163:292-5 (Symposium)
Vague P (*see* Juhan-Vague and Vague). 1990;163:313-5 (Symposium)
Valdiserri A (*see* Filicori et al). 1990;163:1737-42 (Symposium)
Valea FA (*see* Thorp et al). 1990;163:655-6
Valente M (*see* Mastroiacovo et al). 1990;163:695 (Letter)
Valle RF, Sciarra JJ. Ultrasonography's role in detection of intrauterine synechiae. 1990;163:1106-7 (Letter reply)
Van Assche FA. Anencephalics as organ donors. 1990;163:599-600
van Dam PA, Shepherd JH, Lowe DG, Watson JV. Flow cytometric quantitation of tumor-associated antigens in solid tumors. 1990;163:698-9 (Letter)
van der Kwast TH (*see* Leiberman et al). 1990;163:1685-8
van der Mooren K (*see* van Eyck et al). 1990;163:558-66
van Eyck J, Stewart PA, Wladimiroff JW. Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy. 1990;163:1239-42
——, van der Mooren K, Wladimiroff JW. Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development. 1990;163:558-66
Vangsted A (*see* Fuchs et al). 1990;163:1961-7
Vankrieken L (*see* Thomas and Vankrieken). 1990;163:1404-9 (Symposium)
Van Le L (*see* Stern et al). 1990;163:176-7
van Lent M (*see* Akkersdijk et al). 1990;163:591-3
van Lier W (*see* Bergink et al). 1990;163:2132-7 (Symposium)
van Loon AJ, Mantingh A, Thijn CJP, Mooyaart EL. Pelvimetry by magnetic resonance imaging in breech presentation. 1990;163:1256-60
van Roosmalen J. Safe motherhood: Cesarean section or symphsiotomy? 1990;163:1-4 (Clin. opinion)
Van Voorhis BJ, Huettner PC, Clark MR, Hill JA. Immunohistochemical localization of prostaglandin H synthase in the female reproductive tract and endometriosis. 1990;163:57-62
van Vroonhoven CCJ (*see* Leiberman et al). 1990;163:1685-8
Varizi ND (*see* Elias and Varizi). 1990;163:245 (Letter)
Varner MW (*see* Digre et al). 1990;163:1848-52
Varner RE. Retropubic long-needle suspension procedures for stress urinary incontinence. 1990;163:551-7
Vasquez JM, Magargee SF, Kunze E, Hammerstedt RH. Lectins and heparin-binding features of human spermatozoa as analyzed by flow cytometry. 1990;163:2006-12
Vaughn AJ (*see* Johnston et al). 1990;163:743-7
Veldhuis JD (*see* Sollenberger et al). 1990;163:1529-34
Vercellini P (*see* Rovati et al). 1990;163:1490-2
Verlinsky Y (*see* Ginsberg et al). 1990;163:1186-90
Verma RS (*see* Sindwani and Verma). 1990;163:1713-4 (Letter)
Vermesh M (*see* Levin et al). 1990;163:543-4
Vessey MP (*see* Thorogood and Vessey). 1990;163:274-81 (Symposium)
Vianey-Liaud C (*see* George et al). 1990;163:1203-4
Vick DJ (*see* Ferguson et al). 1990;163:926-31
Vijan S (*see* Gagnon et al). 1990;163:1894-9

Vijod AG (*see* Cassidenti et al). 1990;163:1953-60
Vijod MA (*see* Cassidenti et al). 1990;163:1953-60
Villar J, de Onis M, Kestler E, Bolaños F, Cerezo R, Bernedes H. The differential neonatal morbidity of the intrauterine growth retardation syndrome. 1990;163:151-7
——, Repke JT. Calcium supplementation during pregnancy may reduce preterm delivery in high-risk populations. 1990;163:1124-31
Villeneuve MG, Khalifé S, Marcoux S, Blanchet P. Surgical staples in cesarean section: a randomized controlled trial. 1990;163:1641-6
Vintzileos AM (*see* Rodis et al). 1990;163:1168-71
—— (*see* Salafia and Vintzileos). 1990;163:1282-93 (Curr. dev.)
Visser GHA (*see* Ribbert et al). 1990;163:569-71
Voortman G (*see* Bergink et al). 1990;163:2132-7 (Symposium)
Voto LS (*see* Riveros et al). 1990;163:704-5 (Letter)
Vuchetich M (*see* McGregor et al). 1990;163:1580-91

W

Wald NJ (*see* Haddow et al). 1990;163:1372 (Letter)
Waldee JK (*see* Silver et al). 1990;163:917-22
Walker JJ (*see* Cameron et al). 1990;163:931-4
Wallenburg HCS (*see* Akkersdijk et al). 1990;163:591-3
—— (*see* Leiberman et al). 1990;163:1685-8
Walsh JH (*see* Minkoff et al). 1990;163:1598-604
Walsh SW (*see* Dickinson et al). 1990;163:1831-5
—— (*see* Roth et al). 1990;163:1828-30
Walther FJ (*see* Kjos et al). 1990;163:898-903
Walton JC (*see* Cheung et al). 1990;163:1445-9
Wanty SD (*see* Andersen et al). 1990;163:859-67
Ward DC (*see* Grifo et al). 1990;163:2013-9
Warren WB (*see* Matera et al). 1990;163:797-801
Warsof SL (*see* Kuhlmann et al). 1990;163:1264-70
Waston N (*see* Studd et al). 1990;163:1474-9
Watanabe H (*see* Koike et al). 1990;163:696 (Letter)
Watson JV (*see* van Dam et al). 1990;163:698-9 (Letter)
Watson WJ, Thorp JM Jr, Miller RC, Chescheir NC, Katz VL, Seeds JW. Prenatal diagnosis of laryngeal atresia. 1990;163:1456-7
Waxman M (*see* Kniss et al). 1990;163:1883-90
Weaver DS (*see* Jayo et al). 1990;163:614-8
Weiner CP. Cordocentesis and immune thrombocytopenia—continued. 1990;163:1371-2 (Letter)
——, Grose C. Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation from amniotic fluid. 1990;163:1253-5
—— (*see* Grose and Weiner). 1990;163:447-50 (Clin. opinion)
—— (*see* Pollack et al). 1990;163:787-94
Weissman A, Jakobi P. Credit neonatal or obstetric care? 1990;163:1716 (Letter)
Wentworth R (*see* Copel et al). 1990;163:751-6
Werner A (*see* Mallmann et al). 1990;163:1367 (Letter)
Werner AL (*see* Kuhlmann et al). 1990;163:1264-70
Wernley JA (*see* Izquierdo et al). 1990;163:584-7
Wettstein RM. Surrogate parenting contracts. 1990;163:679-80 (Letter)
Whetham J (*see* Cullen et al). 1990;163:466-76
Whitcomb RW (*see* Crowley and Whitcomb). 1990;163:1752-8 (Symposium)
Whitted WA (*see* Strickland et al). 1990;163:262-3 (Letter)
Wians FH Jr (*see* Strickland et al). 1990;163:262-3 (Letter)
Widhalm R (*see* Loewit et al). 1990;163:708-10 (Letter)
Wieacker P (*see* Breckwoldt et al). 1990;163:2213-6 (Symposium)
Wilansky DL, Greisman B. Early hypothyroidism in patients with menorrhagia. 1990;163:697 (Letter reply)
——, ——. Screening infertile women for subclinical hypothyroidism. 1990;163:263 (Letter reply)
Wilcox G (*see* Giles et al). 1990;163:1713 (Letter)
Wild RA, Bartholomew M, Applebaum-Bowden D, Demers LM, Hazzard W, Santen RJ. Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women. 1990;163:1998-2005
Willett WC (*see* Milunsky et al). 1990;163:693 (Letter reply)
—— (*see* Stampfer et al). 1990;163:285-91 (Symposium)
Williams LM (*see* McColgin et al). 1990;163:1503-5
Willoughby A (*see* Minkoff et al). 1990;163:1598-604
Wilson L Jr, Parsons MT, Flouret G. Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist. 1990;163:1875-82
——, ——, Ouano L, Flouret G. A new tocolytic agent: development of an oxytocin antagonist for inhibiting uterine contractions. 1990;163:195-202
Wilson RD (*see* Gilchrist et al). 1990;163:692-3 (Letter)
Winston RML (*see* Dimitry et al). 1990;163:244-5 (Letter)
Wisdom S (*see* Cameron et al). 1990;163:931-4
Wise GJ (*see* Goldstein et al). 1990;163:589-91
Wiskind AK, Toledo AA, Dudley AG, Zusmanis K. Adhesion formation after ovarian wound repair in New Zealand white rabbits: a comparison of ovarian microsurgical closure with ovarian nonclosure. 1990;163:1674-8
Witkin SS (*see* Naz et al). 1990;163:610-3
Witter FR, Shirey RS, Nicol SL, Ness PM. Postinjection kinetics of antepartum Rh immune globulin. 1990;163:784-6
Wladimiroff JW (*see* van Eyck et al). 1990;163:558-66
—— (*see* van Eyck et al). 1990;163:1239-42
Woessner JF Jr (*see* Rajabi et al). 1990;163:499-503
Wolfe WM (*see* Pasic and Wolfe). 1990;163:587-8
Woodard DS (*see* Dombroski et al). 1990;163:1938-43
Woods JR Jr (*see* Plessinger and Woods). 1990;163:1659-64
—— (*see* Sherer et al). 1990;163:1092 (Letter reply)
—— (*see* Sites et al). 1990;163:1630-1
—— (*see* Thompson et al). 1990;163:986-95
Worthington BS (*see* Johnson et al). 1990;163:601-7
Wright C (*see* Stewart et al). 1990;163:1196-9
Wright JW, Patterson RM, Ridgway LE III, Berkus MD. Effect of tocolytic agents on fetal umbilical velocimetry. 1990;163:748-50
——, Ridgway LE. Sources of variability in umbilical artery systolic/diastolic ratios: implications of Poiseuille equation. 1990;163:1788-91
——, Ridgway LE III, Patterson RM. Adjusting the loading dose of magnesium sulfate for tocolysis. 1990;163:889-92
—— (*see* Ridgway et al). 1990;163:879-82
Wynn V (*see* Godsland et al). 1990;163:348-53 (Symposium)

X

Xu L, Chang V, Murphy A, Rock JA, Damewood M, Schlaff W, Zacur HA. Antinuclear antibodies in sera of patients with recurrent pregnancy wastage. 1990;163:1493-7

Y

Yagel S (*see* Ben-Chetrit et al). 1990;163:1161-3
Yamada T (*see* Tabor et al). 1990;163:669-76
Yap AS, Clouston WM, Mortimer RH, Drake RF. Acromegaly first diagnosed in pregnancy: the role of bromocriptine therapy. 1990;163:477-8
Yarali H (*see* Durukan et al). 1990;163:594-5
Yeast JD (*see* Thorp et al). 1990;163:607-9
Yen SSC (*see* Jaffe and Yen). 1990;163:1719-20 (Symposium)

Yeomans ER, Lowe TW, Eigenbrodt EH, Cunningham FG. Liver histopathologic findings in women with sickle cell disease given prophylactic transfusion during pregnancy. 1990;163:958-64
—— (*see* Strickland et al). 1990;163:4-8 (Clin. opinion)
Yetman TJ. Vaginal birth after cesarean section: an appraisal of the reappraisal. 1990;163:692 (Letter reply)
Yoong AFE. Laparoscopic ventrosuspensions. 1990;163:1151-3
Yoshida Y (*see* Manabe and Yoshida). 1990;163:52-6
Yoshizato T (*see* Horimoto et al). 1990;163:1480-4
Young BK (*see* Frieden et al). 1990;163:830-3
—— (*see* Hoskins et al). 1990;163:1944-7
Young JA (*see* Macri et al). 1990;163:1248-53
Yousufuddin M (*see* Smythe et al). 1990;163:1037-9
Yudkin P (*see* Dennis et al). 1990;163:267 (Letter reply)
Yuen BH (*see* Rodway et al). 1990;163:1546-51
Yussman MA (*see* Rao et al). 1990;163:1098-9 (Letter)

Z

Zacur HA (*see* Xu et al). 1990;163:1493-7
Zacut D (*see* Ben-Chetrit et al). 1990;163:1161-3
Zampino G (*see* Mastroiacovo et al). 1990;163:695 (Letter)
Zemel MB (*see* Sowers and Zemel). 1990;163:262 (Letter reply)
Zheng X-Z (*see* Reece et al). 1990;163:162-9 (Curr. dev.)
Zimmer EZ (*see* Goldstein et al). 1990;163:1261-3
Zusmanis K (*see* Wiskind et al). 1990;163:1674-8
Zuspan F (*see* Lenfant et al). 1990;163:2113 (Symposium)
Zwierzina W-D (*see* Kunz et al). 1990;163:417-20 (Symposium)

Subject index*

A

Abdominal delivery; *see* **Cesarean section**
Abdominal wall
The effect of mode of delivery on the perinatal outcome in fetuses with abdominal wall defects (Moretti et al). 1990;163:833-8
Fetal gastroschisis and omphalocele: Is cesarean section the best mode of delivery? (Lewis et al). 1990;163:773-5
Necrotizing fasciitis of the total abdominal wall after sterilization by partial salpingectomy (Cederna et al). 1990;163:138-9
Ultrasonographic assessment of intestinal damage in fetuses with gastroschisis: Is it of clinical value? (Lenke et al). 1990;163:995-8
Abnormalities
Ascertainment bias in diabetic outcome data (FitzSimmons and Davidson) (Letter); (Damm and Mølsted-Pedersen) (Reply). 1990;163:682-3
The associated anomalies that determine prognosis in congenital omphaloceles (Tucci and Bard). 1990;163:1646-9
The association of single umbilical artery with cytogenetically abnormal pregnancies (Saller et al). 1990;163:922-5
The effect of mode of delivery on the perinatal outcome in fetuses with abdominal wall defects (Moretti et al). 1990;163:833-8
Growth of linear parameters in trisomy 18 fetuses (Droste et al). 1990;163:158-61
In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1
Perinatal outcome of forty-nine pregnancies complicated by acardiac twinning (Moore et al). 1990;163:907-12
Prenatal diagnosis of laryngeal atresia (Watson et al). 1990;163:1456-7
Transvaginal ultrasonographic detection of congenital anomalies in the first trimester (Cullen et al). 1990;163:466-76
Abnormalities, animal
Embryofetal effects of pentamidine isethionate administered to pregnant Sprague-Dawley rats (Harstad et al). 1990;163:912-6
Abortion
Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? (Chervenak and McCullough). 1990;163:1425-9 (Clin. opinion)
One case of 45,X abortus with high CA 125 level (Check). 1990;163:1717 (Letter)
Postmortem chorionic villus sampling is a better method for cytogenetic evaluation of early fetal loss than culture of abortus material (Johnson et al). 1990;163:1505-10
Prediction of residual trophoblastic tissue in first-trimester abortions: the role of transvaginal Doppler ultrasonography (Reuwen). 1990;163:2024-5 (Letter)
Pregnancy loss terminology (Branch and Scott) (Letter); (Pridjian and Moawad) (Reply). 1990;163:245-6
A progesterone-induced blocking factor corrects high resorption rates in mice treated with antiprogesterone (Szekeres-Bartho et al). 1990;163:1320-1
Recurrent adverse pregnancy outcome and antiphospholipid antibodies (Reece et al). 1990;163:162-9 (Curr. dev.)
Abortion, habitual
Antinuclear antibodies in sera of patients with recurrent pregnancy wastage (Xu et al). 1990;163:1493-7
Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss (Branch et al). 1990;163:210-6
Serum levels of interleukin-2 and tumor necrosis factor α in women with recurrent abortion (Mallmann et al). 1990;163:1367 (Letter)
Sharing of human leukocyte antigens in primary and secondary recurrent spontaneous abortions (Ho et al). 1990;163:178-88
Abortion, induced
Morphologic study of changes of collagenous tissue in the amnion and cervix during prostaglandin-induced abortion and delivery (Manabe and Yoshida). 1990;163:52-6

*July, part 1, 1-272; part 2, 273-446; August, 447-714; September, 715-1108; October, part 1, 1109-1378; part 2, 1379-1420; November, part 1, 1421-1718; part 2, 1719-1770; December, part 1, 1771-2112; part 2, 2113-218.

Abortion, legal
A chill wind blows: Webster, obstetrics, and the health of women (Chavkin and Rosenfield). 1990;163:450-2 (Clin. opinion)
Abortion, missed
Early pregnancy failure—appropriate terminology (Goldstein). 1990;163:1093 (Letter)
Pregnancy loss terminology (Branch and Scott) (Letter); (Pridjian and Mcawad) (Reply). 1990;163:245-6
Abortion, threatened
A case of Rh isoimmunization: Should threatened first-trimester abortion be an indication for Rh immune globulin prophylaxis? (Dayton et al). 1990;163:63-4
Accelerated fetal growth; *see* **Macrosomia**
Access, venous; *see* **Catheters, indwelling**
Acid-base equilibrium
A comparison of the effect of epidural, general, and no anesthesia on funic acid-base values by stage of labor and type of delivery (Shyken et al). 1990;163:802-7
Comparison of umbilical artery pH and 5-minute Apgar score in the low-birth-weight and very-low-birth-weight infant (Stark et al). 1990;163:818-23
Acidemia, fetal; *see* **Fetal distress**
Acidosis, animal
Does the umbilical artery systolic/diastolic ratio reflect flow or acidosis? (Copel et al). 1990;163:751-6
Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1990;163:1313-20
Acidosis, diabetic
Diabetic ketoacidosis and insulin resistance with subcutaneous terbutaline infusion: a case report (Tibaldi et al). 1990;163:509-10
Acoustic stimulation
The intrauterine sound environment of the human fetus during labor (Benzaquen et al). 1990;163:484-90
Vibroacoustic stimulation and fetal behavioral state in normal term human pregnancy (Devoe et al). 1990;163:1156-61
Acquired immunodeficiency syndrome
Transplacental transfer of zidovudine in the near-term pregnant baboon (Hankins et al). 1990;163:728-32
Acromegaly
Acromegaly first diagnosed in pregnancy: the role of bromocriptine therapy (Yap et al). 1990;163:477-8
Activated partial thromboplastin time; *see* **Partial thromboplastin time**
Adenocarcinoma
Colon cancer in pregnancy with elevated maternal serum α-fetoprotein level at presentation (Gonsoulin et al). 1990;163:1172-3
Adenomyosis; *see* **Endometriosis**
Adenosine cyclic monophosphate
Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast (Rodway et al). 1990;163:1546-51
Adhesions
Ultrasonography's role in detection of intrauterine synechiae (Stephens) (Letter); (Valle and Sciarra) (Reply). 1990;163:1106-7
Adhesions, animal
Adhesion formation after ovarian wound repair in New Zealand white rabbits: a comparison of ovarian microsurgical closure with ovarian nonclosure (Wiskind et al). 1990;163:1674-8
Adjuvants, immunologic
A progesterone-induced blocking factor corrects high resorption rates in mice treated with antiprogesterone (Szekeres-Bartho et al). 1990;163:1320-1
Adnexal diseases
Management of selected cystic adnexal masses in postmenopausal women by operative laparoscopy: a pilot study (Parker and Berek). 1990;163:1574-7
Adnexitis
Clindamycin dosage scheduling for acute pelvic infection (Chatwani et al). 1990;163:240 (Letter)
Sexually transmitted diseases and human immunodeficiency virus infection among women with pelvic inflammatory disease (Hoegsberg et al). 1990;163:1135-9
Adrenal cortex hormones
Dose response of thyrotropin-releasing hormone on pulmonary maturation in corticosteroid-treated preterm rabbits (Tabor et al). 1990;163:669-76
Adrenergic agents; *see* **Sympathomimetics**
Adrenergic alpha receptor agonists
Effects of clonidine on breathing movements and electrocortical activity in the fetal lamb (Bamford et al). 1990;163:661-8
Adrenergic beta receptor blockaders
β-Adrenergic receptor blockade in the management of pregnant women with mitral stenosis (Al Kasab et al). 1990;163:37-40
Adrenocorticotropic hormone
The effect of adrenocorticotropic hormone infusion on subsequent pituitary response in the sheep fetus (Cuthrell et al). 1990;163:170-4
Mechanism of action of interleukin-1β in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells (Petraglia et al). 1990;163:1307-12
Aerobic exercise; *see* **Exercise**
Aged
Prevalence and determinants of estrogen replacement therapy in elderly women (Cauley et al). 1990;163:1438-44
Aging
Parameters of hypothalamic aging (Seifer and Jones) (Letter); (Alexander and Aksel) (Reply). 1990;163:1715
Air embolism; *see* **Embolism, air**
Alcoholism
Use of the T-ACE questions to detect risk-drinking (Elliot) (Letter); (Sokol) (Reply). 1990;163:684-5
Alkaline phosphatase
Pregnancy-induced hypertension and acute fatty liver of pregnancy: atypical presentations (Brown et al). 1990;163:1154-6
Alpha fetoproteins
Colon cancer in pregnancy with elevated maternal serum α-fetoprotein level at presentation (Gonsoulin et al). 1990;163:1172-3
The effect of adjusting maternal serum α-fetoprotein levels for maternal weight in pregnancies with fetal open spina bifida (Johnson et al). 1990;163:9-11
Maternal serum α-fetoprotein levels in pregnancies complicated by diabetes: implications for screening programs (Martin et al). 1990;163:1209-16
Maternal serum Down syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin (Macri et al). 1990;163:1248-53
Maternal serum α-fetoprotein in pregnancy (Gilchrist et al) (Letter); (Milunsky et al) (Reply). 1990;163:692-3

Alpha fetoproteins—cont'd
Maternal serum α-fetoprotein level useful screening test (Lao). 1990;163:240 (Letter)
The relationship between maternal serum and amniotic fluid α-fetoprotein in women undergoing early amniocentesis (Brumfield et al). 1990;163:903-6
Should amniocenteses always accompany unexplained high maternal serum α-fetoprotein levels? Who decides: public agency, patient, or private physician? (Stephens) (Letter); (Hogge et al) (Reply). 1990;163:697-8
Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level (Jauniaux et al). 1990;163:1558-60
Alprostadil
Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb (Paulick et al). 1990;163:1357-63
Ambulatory care
Outpatient obstetric management of women with type I diabetes (Bourgeois and Duffer). 1990;163:1065-73
Amenorrhea
Autogenic masculinization (Punch and Ansbacher). 1990;163:114-6
Efficacy and safety of intravenous pulsatile gonadotropin-releasing hormone: Lutrepulse for injection (Santoro). 1990;163:1759-64 (Supplement)
Hypothalamic amenorrhea: clinical perspectives, pathophysiology, and management (Liu). 1990;163:1732-6 (Supplement)
Practical aspects of pulsatile gonadotropin-releasing hormone administration (Schriock). 1990;163:1765-70 (Supplement)
American Journal of Obstetrics and Diseases of Women and Children
The first seventy years. 1990;163:1109-10 (Editorial)
American Journal of Obstetrics and Gynecology
The first seventy years. 1990;163:1109-10 (Editorial)
Aminobutyric acids
Zinc, preeclampsia, and γ-aminobutyric acid (Brophy). 1990;163:242-3 (Letter)
Aminoglycoside, toxicity
Cefotetan and the lack of associated bleeding (Martens and Faro) (Letter); (Briggs et al) (Reply). 1990;163:251-2
Amniocentesis; *see also* **Amniotic fluid**
Absence of evidence of pulmonary maturity at amniocentesis in term infants of diabetic mothers (Ojomo and Coustan). 1990;163:954-7
Antenatal diagnosis of molybdenum cofactor deficiency (George et al). 1990;163:1203-4
Detection of congenital toxoplasmosis by chorionic villus sampling and early amniocentesis (Foulon et al). 1990;163:1511-3
The diagnostic values of concurrent nonstress testing, amniotic fluid measurement, and Doppler velocimetry in screening a general high-risk population (Devoe et al). 1990;163:1040-8
Effect of maternal-fetal movement on concentration of cells obtained at genetic amniocentesis (Carlan et al). 1990;163:490-3
Gram stain results from amniocentesis in patients with preterm permature rupture of membranes—comparison of maternal and fetal characteristics (Asrat et al). 1990;163:887-9
Intraamniotic infection in the very early phase of the second trimester (Goldstein et al). 1990;163:1261-3
Intrauterine *Listeria* infection: prenatal diagnosis by biophysical assessment and amniocentesis (Liner). 1990;163:1596-7
Postmortem chorionic villus sampling is a better method for cytogenetic evaluation of early fetal loss than culture of abortus material (Johnson et al). 1990;163:1505-10
The relationship between maternal serum and amniotic fluid α-fetoprotein in women undergoing early amniocentesis (Brumfield et al). 1990;163:903-6
Should amniocenteses always accompany unexplained high maternal serum α-fetoprotein levels? Who decides: public agency, patient, or private physician? (Stephens) (Letter); (Hogge et al) (Reply). 1990;163:697-8
The stuck twin phenomenon: Ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses (Mahony et al). 1990;163:1513-22
Transcervical chorionic villus sampling and amniocentesis: a comparison of reliability, culture findings, and fetal outcome (Ferguson et al). 1990;163:926-31
Amnioinfusion; *see* **Amniotic fluid**
Amnion
Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells (Kniss et al). 1990;163:1883-90
Morphologic study of changes of collagenous tissue in the amnion and cervix during prostaglandin-induced abortion and delivery (Manabe and Yoshida). 1990;163:52-6
Amniotic fluid; *see also* **Amniocentesis**
Amnioinfusion and prevention of meconium aspiration syndrome (Falciglia) (Letter); (Sadovsky and Amon) (Reply). 1990;163:258-9
Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor (Romero et al). 1990;163:968-74
Amniotic fluid midtrimester delta optical density measurements at 450 nm in Rh disease (Nelson) (Letter); (Queenan) (Reply). 1990;163:710-2
Comparisons of glycosylated and nonglycosylated amniotic fluid prolactins in normal second- and third-trimester pregnancies (McCoshen et al). 1990;163:81-5
Corticotropin-releasing hormone in amniotic fluid (Laatikainen) (Letter); (Sasaki) (Reply). 1990;163:1102-3
Dehydration-induced oligohydramnios (Ross) (Letter); (Sherer et al) (Reply). 1990;163:1091-2
Detection of fetal blood contamination by amniotic fluid obtained during cordocentesis (Lazebnik et al). 1990;163:78-80
Does prophylactic amnioinfusion make a difference? (Chauhan) (Letter); (Strong et al) (Reply). 1990;163:1370-1
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66
The effect of amnioinfusion on uterine pressure and activity: a preliminary report (Posner et al). 1990;163:813-8
In vitro inhibition of esterase activity in amniotic fluid: comparison with bacterial cultures (Hoskins et al). 1990;163:1944-7
Infants of diabetic mothers with accelerated fetal growth by ultrasonography: Are they all alike? (Keller et al). 1990;163:893-7
Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in twin gestations with preterm labor (Romero et al). 1990;163:757-61
Intraamniotic infection in the very early phase of the second trimester (Goldstein et al). 1990;163:1261-3

Normal amniotic pressure in oligohydramnios after preterm rupture of membranes (Johnson and Maxwell) (Letter); (Nicolini et al) (Reply). 1990;163:1103-4
Observations on the cause of oligohydramnios in prolonged pregnancy (Trimmer et al). 1990;163:1900-3
Perinatal survival with expectant management of midtrimester rupture of membranes (Major and Kitzmiller). 1990;163:838-44
Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation from amniotic fluid (Weiner and Grose). 1990;163:1253-5
Preterm delivery: a risk factor for retained placenta (Romero et al). 1990;163:823-5
The relationship between maternal serum and amniotic fluid α-fetoprotein in women undergoing early amniocentesis (Brumfield et al). 1990;163:903-6
Risk factors for perinatal group B streptococcal disease after amniotic fluid colonization (Silver et al). 1990;163:19-25
The stuck twin phenomenon: Ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses (Mahony et al). 1990;163:1513-22
Superiority of the four-quadrant sum over the single-deepest-pocket technique in ultrasonographic identification of abnormal amniotic fluid volumes (Moore). 1990;163:762-7

Amniotic fluid embolism; *see* **Embolism, amniotic fluid**

Amphetamines
The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening (Gillogley et al). 1990;163:1535-42
Prevalence of use of cocaine and other substances in an obstetric population (Matera et al). 1990;163:797-801

Analgesia, epidural
Epidural analgesia and cesarean section for dystocia (Chestnut) (Letter); (Thorp and Parisi) (Reply). 1990;163:247-9
Labor epidural analgesia and dystocia-related cesarean section (Downing and Knape). 1990;163:1096-7 (Letter)
The local anesthetic dose is an important issue in evaluation of effects of epidural analgesia in nulliparous women (Puppa et al) (Letter); (Thorp) (Reply). 1990;163:701-3

Androgens
Discrepancy in the diagnoses of hydatidiform mole by macroscopic and microscopic findings and the deoxyribonucleic acid fingerprint method (Takahashi et al). 1990;163:112-3

Anemia
Is intrauterine transfusion associated with diminished fetal growth? (Utter et al). 1990;163:1781-4

Anemia, sickle cell
Liver histopathologic findings in women with sickle cell disease given prophylactic transfusion during pregnancy (Yeomans et al). 1990;163:958-64

Anencephaly
Anencephalics as organ donors (Van Assche). 1990;163:599-600

Anesthesia
Can anesthetic-related maternal mortality be reduced? (Conklin). 1990;163:253-4 (Letter)
A comparison of the effect of epidural, general, and no anesthesia on funic acid-base values by stage of labor and type of delivery (Shyken et al). 1990;163:802-7
Effect of uterine contractility and maternal hypotension on prolonged decelerations after bupivacaine epidural anesthesia (Steiger and Nageotte). 1990;163:808-12
Effectiveness of 1% lidocaine dorsal penile nerve block in infant circumcision (Arnett et al). 1990;163:1074-80

Aneuploidy
Correlation of the testicular determinant factor sequence zinc finger Y with varying gonadal phenotypes in a series of 13 subjects with gonadal dysgenesis because of Y aneuploidy (Tho et al). 1990;163:1968-75

Aneurysm, cerebral; *see* **Cerebral aneurysm**

Angiomatosis
Systemic cystic angiomatosis in pregnancy: a case presentation and review of the literature (Bardeguez et al). 1990;163:42-5

Angiotensin
Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome (Brown et al). 1990;163:1853-61

Angiotensin binding sites; *see* **Receptors, angiotensin**

Anorexia nervosa
Infertility and eating disorders (Stewart et al). 1990;163:1196-9

Anoxia
The effect of maternal hyperoxia on behavioral activity in growth-retarded human fetuses (Gagnon et al). 1990;163:1894-9

Antibiotics
Antibiotic therapy in preterm premature rupture of membranes: a randomized, prospective, double-blind trial (Johnston et al). 1990;163:743-7
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91
Placental transfer of cefazolin and piperacillin in pregnancies remote from term complicated by Rh isoimmunization (Brown et al). 1990;163:938-43

Antibodies
Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84
Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions. III. Cytotoxic potential in vitro and characterization of antigen(s) (Kutteh et al). 1990;163:1301-6
A comparison of sperm antibody assays (Daru and Mathur). 1990;163:1622-9

Antibodies, antinuclear
Antinuclear antibodies in sera of patients with recurrent pregnancy wastage (Xu et al). 1990;163:1493-7

Antibodies, monoclonal
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84

Antibody formation
Antisperm antibodies in women undergoing intrauterine insemination (Goldberg et al). 1990;163:65-8
Digoxin-like immunoreactive factor and respiratory distress syndrome (Goodlin). 1990;163:1091 (Letter)
Recurrent adverse pregnancy outcome and antiphospholipid antibodies (Reece et al). 1990;163:162-9 (Curr. dev.)

Antibody formation, animal
Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a

Antibody formation, animal—cont'd
model for autoimmune fetal loss (Branch et al). 1990;163:210-6
Anticardiolipin antibody; *see* **Cardiolipins**
Antiestrogens; *see* **Estrogen antagonists**
Antigenic determinants
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84
Antigens
A comparison of sperm antibody assays (Daru and Mathur). 1990;163:1622-9
Lymphocyte proliferative response to fertilization antigen in patients with antisperm antibodies (Naz et al). 1990;163:610-3
Antigens, neoplasm
Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions. III. Cytotoxic potential in vitro and characterization of antigen(s) (Kutteh et al). 1990;163:1301-6
Flow cytometric quantitation of tumor-associated antigens in solid tumors (van Dam et al) (Letter); (Nozawa) (Reply). 1990;163:698-9
Antigens, tumor-associated, carbohydrate
Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm (Hardardottir et al). 1990;163:1925-31
One case of 45,X abortus with high CA 125 level (Check). 1990;163:1717 (Letter)
Preoperative evaluation of serum CA 125, TAG 72, and CA 15-3 in patients with endometrial carcinoma (Soper et al). 1990;163:1204-9
The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemotherapy after surgical cytoreduction (Hunter et al). 1990;163:1164-7
The use of serial CA 125 concentrations to monitor endometriosis in infertile women (Pittaway). 1990;163:1032-7
Antihypertensive agents
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. 1990;163:1691-712 (Supplement)
Antineoplastic agents
Expression of P-glycoprotein in epithelial ovarian cancer: evaluation as a marker of multidrug resistance (Rubin et al). 1990;163:69-73
Antinuclear antibodies; *see* **Antibodies, antinuclear**
Antioxidants
Smoking and the antioxidant ascorbic acid: plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women (Basu et al). 1990;163:1948-52
Antiphosphatidylserine antibody; *see* **Phosphatidylserines**
Antiphospholipid antibodies; *see* **Phospholipids**
Antiprogesterone; *see* **Mifepristone**
Antiprogesterone, animal; *see* **Receptors, progesterone, animal**
Antisperm antibodies; *see* **Spermatozoa**
Antithrombin III, deficiency
Emergency treatment with recombinant tissue plasminogen activator of pulmonary embolism in a pregnant woman with antithrombin III deficiency (Baudo et al). 1990;163:1274-5
Aorta, descending; *see* **Aorta, thoracic**
Aorta, thoracic
Blood flow velocity and pulsatile diameter changes in the fetal descending aorta: a longitudinal study (Stale et al). 1990;163:26-9
Apgar score
Comparison of umbilical artery pH and 5-minute Apgar score in the low-birth-weight and very-low-birth-weight infant (Stark et al). 1990;163:818-23
Apocrine glands
Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm (Hardardottir et al). 1990;163:1925-31
Argipressin
Role of vasopressin in mediation of fetal cardiovascular responses to acute hypoxia (Piacquadio et al). 1990;163:1294-300
Aromatase
Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast (Rodway et al). 1990;163:1546-51
Arteriovenous fistula
In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1
Ascites, fetal
Antenatal spontaneous perforation of the extrahepatic biliary tree (Chilukuri et al). 1990;163:1201-2
Ascorbic acid
Smoking and the antioxidant ascorbic acid: plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women (Basu et al). 1990;163:1948-52
Asphyxia, fetal; *see* **Fetal anoxia**
Asphyxia neonatorum
What is birth asphyxia? (Clark and Quirk) (Letter); (Thorp) (Reply). 1990;163:1367-8
Aspirin
Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome (Brown et al). 1990;163:1853-61
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. 1990;163:1691-712 (Supplement)
Atherosclerosis
Effects of oral contraceptives on lipid metabolism (Gevers Leuven et al). 1990;163:1410-3 (Supplement)
Oral contraceptives, lipoproteins, and atherosclerosis (Adams et al). 1990;163:1388-92 (Supplement)
Role of plasma lipoproteins in the pathogenesis of atherosclerotic disease, with special reference to sex hormone effects (Tikkanen). 1990;163:296-304 (Supplement)
Atrial natriuretic factor; *see* **Natriuretic peptides, atrial**
Autoantibodies
Association between autoantibodies and unexplained infertility unproved (Maier) (Letter); (Taylor and Campbell) (Reply). 1990;163:243
Autogenic masculinization; *see* **Self medication**
Autoimmune diseases
Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84
Autoimmunity, animal
Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss (Branch et al). 1990;163:210-6

Autologous blood transfusion; *see* **Blood transfusion, autologous**
Automatic data processing
Blood pressure measured telemetrically from home throughout pregnancy (Mooney et al). 1990;163:30-6

B

Bacterial infections
Antenatal microbiologic and maternal risk factors associated with prematurity (McGregor et al). 1990;163:1465-73
Bacterial vaginosis: diagnostic and pathogenetic findings during topical clindamycin therapy (Livengood et al). 1990;163:515-20
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91
A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4
In vitro inhibition of esterase activity in amniotic fluid: comparison with bacterial cultures (Hoskins et al). 1990;163:1944-7
Bacterial infections, animal
A rabbit model for bacteria-induced preterm pregnancy loss (Dombroski et al). 1990;163:1938-43
Behavior, fetus
Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy (van Eyck et al). 1990;163:1239-42
Vibroacoustic stimulation and fetal behavioral state in normal term human pregnancy (Devoe et al). 1990;163:1156-61
Behavior, infant
The association of intrapartum asphyxia in the mature fetus with newborn behavior (Low et al). 1990;163:1131-5
Correlations between antepartum maternal metabolism and newborn behavior (Rizzo et al). 1990;163:1458-64
Beta-adrenergic receptor blockaders; *see* **Adrenergic beta receptor blockaders**
17β-estradiol; *see* **Estradiol**
Bias
Ascertainment bias in diabetic outcome data (FitzSimmons and Davidson) (Letter); (Damm and Mølsted-Pedersen) (Reply). 1990;163:682-3
Biliary tract
Antenatal spontaneous perforation of the extrahepatic biliary tree (Chilukuri et al). 1990;163:1201-2
Biophysical assessment; *see* **Fetal monitoring**
Biopsy
Preembryo biopsy and analysis of blastomeres by in situ hybridization (Grifo et al). 1990;163:2013-9
Preventing cervical conization by achieving satisfactory colposcopy with hygroscopic cervical dilators (Stern et al). 1990;163:176-7
Second-trimester placental biopsy for rapid fetal karyotyping (Cameron et al). 1990;163:931-4
Birth weight
The effect of physical activity during pregnancy on preterm delivery and birth weight (Klebanoff et al). 1990;163:1450-6
The effect of race on the relationship between fetal death and altered fetal growth (Ferguson and Myers). 1990;163:1222-30
Neonatal morphometrics after endurance exercise during pregnancy (Clapp and Capeless). 1990;163:1805-11
Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33
Ultrasonographic fetal weight estimation by an integrated computer-assisted system: Can each laboratory improve its accuracy? (Thompson et al). 1990;163:986-95
Bladder
The effect of large uterine fibroids on urinary bladder function symptoms (Langer et al). 1990;163:1139-41
Blastomeres
Preembryo biopsy and analysis of blastomeres by in situ hybridization (Grifo et al). 1990;163:2013-9
Bleeding, first-trimester; *see* **Abortion, threatened**
Blood, radiation effects
Hyperkalemia secondary to irradiation of packed red blood cells: possible effects with intravascular fetal transfusion (Thorp et al). 1990;163:607-9
Blood coagulation
Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease (Kelleher). 1990;163:392-5 (Supplement)
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Disorders of the hemostatic system and the risk of the development of thrombotic and cardiovascular diseases: limitations of laboratory diagnosis (Kluft). 1990;163:305-12 (Supplement)
Effects of long-term gestodene-containing oral contraceptive administration on hemostasis (Abbate et al). 1990;163:424-9 (Supplement)
Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid (Jespersen et al). 1990;163:396-403 (Supplement)
Enhanced generation and resolution of fibrin in women above the age of 30 years using oral contraceptives low in estrogen (Gram et al). 1990;163:438-42 (Supplement)
Hemostasis profile in women taking low-dose oral contraceptives (David et al). 1990;163:420-3 (Supplement)
Influence of oral contraceptives on coagulation tests in native blood and plasma (Kunz et al). 1990;163:417-20 (Supplement)
Metabolic changes during the normal menstrual cycle: a longitudinal study (Lebech et al). 1990;163:414-6 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Blood coagulation disorders
Disorders of the hemostatic system and the risk of the development of thrombotic and cardiovascular diseases: limitations of laboratory diagnosis (Kluft). 1990;163:305-12 (Supplement)
Blood flow velocity
Blood flow velocity and pulsatile diameter changes in the fetal descending aorta: a longitudinal study (Stale et al). 1990;163:26-9
Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy (van Eyck et al). 1990;163:1239-42
Blood gas analysis
Relationship of fetal biophysical profile and blood gas values

Blood gas analysis—cont'd
at cordocentesis in severely growth-retarded fetuses (Ribbert et al). 1990;163:569-71
Blood platelet disorders
Responsibility expands to extended family members (Saving et al). 1990;163:1369-70 (Letter)
Blood platelets
Changes in platelet angiotensin II binding sites associated with the human menstrual cycle (Baker et al). 1990;163:175-6
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Cordocentesis and immune thrombocytopenia—continued (Weiner). 1990;163:1371-2 (Letter)
Hemostasis profile in women taking low-dose oral contraceptives (David et al). 1990;163:420-3 (Supplement)
Blood pressure
Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive value for pregnancy-induced hypertension (Hume et al). 1990;163:826-9
Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome (Brown et al). 1990;163:1853-61
Blood pressure, animal
Does the umbilical artery systolic/diastolic ratio reflect flow or acidosis? (Copel et al). 1990;163:751-6
Blood pressure determination
Blood pressure measured telemetrically from home throughout pregnancy (Mooney et al). 1990;163:30-6
Blood transfusion
Liver histopathologic findings in women with sickle cell disease given prophylactic transfusion during pregnancy (Yeomans et al). 1990;163:958-64
Blood transfusion, autologous
A reappraisal of the need for autologous blood donation in the obstetric patient (Andres et al). 1990;163:1551-3
Blood transfusion, intrauterine
Acute fetal hemodynamic alterations after intrauterine transfusion for treatment of severe red cell alloimmunization (Moise et al). 1990;163:776-84
Continuing controversy in alloimmune thrombocytopenia: fetal hyperimmunoglobulinemia fails to prevent thrombocytopenia (Nicolini et al). 1990;163:1144-6
Effects of intravascular fetal blood transfusion on fetal intracardiac Doppler velocity waveforms (Rizzo et al). 1990;163:1231-8
Hyperkalemia secondary to irradiation of packed red blood cells: possible effects with intravascular fetal transfusion (Thorp et al). 1990;163:607-9
Is intrauterine transfusion associated with diminished fetal growth? (Utter et al). 1990;163:1781-4
Blood viscosity, animal
Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1990;163:1313-20
Blood volume
Interrelationship between atrial natriuretic factor concentrations and acute volume expansion in pregnant and nonpregnant women (Hatjis et al). 1990;163:45-50
Body habitus; *see* **Body mass index**
Body mass index
Glucose tolerance in pregnancy: ethnic variation and influence of body habitus (Green et al). 1990;163:86-92
Body weight
The effect of adjusting maternal serum α-fetoprotein levels for maternal weight in pregnancies with fetal open spina bifida (Johnson et al). 1990;163:9-11
Bone density
The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants (Studd et al). 1990;163:1474-9
Bone loss, age-related; *see* **Osteoporosis**
Books
Books received. 1990;163:713, 2031
Brain
Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry (Ikeda and Mori). 1990;163:494-8
Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors (Devaskar et al). 1990;163:1350-6
Breakthrough bleeding; *see* **Uterine hemorrhage**
Breast diseases
Breast diagnostics in clinical gynecology (Cross) (Letter); (Gleicher) (Reply). 1990;163:686-7
Breast disease programs and mammography training (Dodd and McLelland) (Letter); (Gleicher) (Reply). 1990;163:689-91
Breast milk; *see* **Milk, human**
Breast neoplasms
Oral contraceptives and breast cancer (Schlesselman). 1990;163:1379-87 (Supplement)
Prevalence and determinants of estrogen replacement therapy in elderly women (Cauley et al). 1990;163:1438-44
Breech presentation
Pelvimetry by magnetic resonance imaging in breech presentation (van Loon et al). 1990;163:1256-60
Vaginal breech delivery: a five-year prospective evaluation of a protocol using computed tomographic pelvimetry (Christian et al). 1990;163:848-55
Broad ligament
Papillary cystadenoma of the broad ligament in von Hippel-Lindau disease (Korn et al). 1990;163:596-8
Bromocriptine
Acromegaly first diagnosed in pregnancy: the role of bromocriptine therapy (Yap et al). 1990;163:477-8
Bulimia
Infertility and eating disorders (Stewart et al). 1990;163:1196-9
Bupivacaine
Effect of uterine contractility and maternal hypotension on prolonged decelerations after bupivacaine epidural anesthesia (Steiger and Nageotte). 1990;163:808-12

C

CA 15-3; *see* **Antigens, tumor-associated, carbohydrate**
CA 125; *see* **Antigens, tumor-associated, carbohydrate**
Calcinosis
Prenatal diagnosis of fetal intracranial calcifications (Koga et al). 1990;163:1543-5
Calcium
Calcium supplementation during pregnancy may reduce preterm delivery in high-risk populations (Villar and Repke). 1990;163:1124-31
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. 1990;163:1691-712 (Supplement)

Relationship between plasma calcium fractions, other bone-related variables, and serum follicle-stimulating hormone levels in premenopausal, perimenopausal, and postmenopausal women (Nordin et al). 1990;163:140-5

Calcium channel blockers

Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy (Skajaa et al). 1990;163:1323-33

Candidiasis, vulvovaginal

A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4

Captopril

Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat (Mabie et al). 1990;163:1861-7

Carbohydrates, metabolism

Clinical aspects of the relationship between oral contraceptives, abnormalities in carbohydrate metabolism, and the development of cardiovascular disease (Gaspard and Lefebvre). 1990;163:334-43 (Supplement)

Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: results of a controlled, randomized, prospective study (Kjos et al). 1990;163:1822-7

Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom Study (Simon et al). 1990;163:382-7 (Supplement)

Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease (Juhan-Vague and Vague). 1990;163:313-5 (Supplement)

Low-dose oral contraceptives and carbohydrate metabolism (Godsland et al). 1990;163:348-53 (Supplement)

Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level (Skouby et al). 1990;163:343-8 (Supplement)

Carbon dioxide laser surgery; *see* **Laser surgery**

Carcinogens; *see also* **Teratogens**

Ovarian granulosa cell tumorigenesis in SWR-derived F1 hybrid mice: preneoplastic follicular abnormality and malignant disease progression (Tennent et al). 1990;163:625-34

Carcinoma

Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions. III. Cytotoxic potential in vitro and characterization of antigen(s) (Kutteh et al). 1990;163:1301-6

Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81

Invasive carcinoma of the vulva (Cavanagh et al). 1990;163:1007-15

Preoperative evaluation of serum CA 125, TAG 72, and CA 15-3 in patients with endometrial carcinoma (Soper et al). 1990;163:1204-9

Carcinoma, ovarian; *see* **Ovarian neoplasms**

Cardiac output

Acute fetal hemodynamic alterations after intrauterine transfusion for treatment of severe red cell alloimmunization (Moise et al). 1990;163:776-84

Measurement of cardiac output by impedance technique (Easterling and Benedetti). 1990;163:1104-6 (Letter)

Measurement of cardiac output with impedance cardiography (Lechner). 1990;163:1096 (Letter)

Cardiac-placental connection; *see* **Fetal heart**

Cardiography, impedance

Measurement of cardiac output by impedance technique (Easterling and Benedetti). 1990;163:1104-6 (Letter)

Measurement of cardiac output with impedance cardiography (Lechner). 1990;163:1096 (Letter)

Cardiolipins

Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84

Recurrent adverse pregnancy outcome and antiphospholipid antibodies (Reece et al). 1990;163:162-9 (Curr. dev.)

Cardiomyopathy, congestive

Ritodrine infusion and cardiomyopathy (Caritis) (Letter); (Blickstein) (Reply). 1990;163:254-6

Cardiopulmonary bypass

Sinusoidal fetal heart rate pattern during cardiopulmonary bypass (Burke et al). 1990;163:17-8

Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy (Esposito et al). 1990;163:572-4

Cardiovascular complications of pregnancy; *see* **Pregnancy complications, cardiovascular**

Cardiovascular diseases, complications

Clinical aspects of the relationship between oral contraceptives, abnormalities in carbohydrate metabolism, and the development of cardiovascular disease (Gaspard and Lefebvre). 1990;163:334-43 (Supplement)

Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease (Kelleher). 1990;163:392-5 (Supplement)

Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease (Juhan-Vague and Vague). 1990;163:313-5 (Supplement)

Some aspects of the relationship between oral contraceptives, lipid abnormalities, and cardiovascular disease (Samsioe and Mattsson). 1990;163:354-8 (Supplement)

Cardiovascular diseases, diagnosis

Disorders of the hemostatic system and the risk of the development of thrombotic and cardiovascular diseases: limitations of laboratory diagnosis (Kluft). 1990;163:305-12 (Supplement)

Cardiovascular diseases, epidemiology

An epidemiologic survey of cardiovascular disease in women taking oral contraceptives (Thorogood and Vessey). 1990;163:274-81 (Supplement)

Past use of oral contraceptives and cardiovascular disease: a meta-analysis in the context of the Nurses' Health Study (Stampfer et al). 1990;163:285-91 (Supplement)

Cardiovascular diseases, mortality

Cardiovascular death among women under 40 years of age using low-estrogen oral contraceptives and intrauterine devices in Finland from 1975 to 1984 (Hirvonen and Idänpään-Heikkilä). 1990;163:281-4 (Supplement)

Cardiovascular system

Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system (Beck-Nielsen et al). 1990;163:292-5 (Supplement)

Cardiovascular system, animal
Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes (Plessinger and Woods). 1990;163:1659-64
Carotid artery, internal
Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry (Ikeda and Mori). 1990;163:494-8
Carrier state
HBcAg identification in the placental cytotypes of symptom-free HBsAg-carrier mothers: a study with the immunoperoxidase method (Lucifora et al). 1990; 163:235-9
Catechol estrogens
Formation, metabolism, and physiologic importance of catecholestrogens (Ball and Knuppen). 1990;163:2163-70 (Symposium)
Catecholamines
Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women (Jaffe et al). 1990;163:1727-31 (Supplement)
Catheterization
Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle (Figueroa et al). 1990;163:648-54
Catheters, indwelling
Ultrasonographic guidance for central venous access (Greenspoon) (Letter); (Lee) (Reply). 1990;163: 252
Caucasoid race
The effect of race on the relationship between fetal death and altered fetal growth (Ferguson and Myers). 1990;163:1222-30
Cefazolin
Placental transfer of cefazolin and piperacillin in pregnancies remote from term complicated by Rh isoimmunization (Brown et al). 1990;163:938-43
Cefotetan
Cefotetan and the lack of associated bleeding (Martens and Faro) (Letter); (Briggs et al) (Reply). 1990;163: 251-2
Cell division
Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies (Mutch et al). 1990;163:1920-4
Cell growth; *see* **Cell division**
Cell-mediated immunity; *see* **Immunity, cellular**
Cell surface glycoproteins; *see* **Membrane glycoproteins**
Cells, cultured
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163: 1932-8
Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells (Kniss et al). 1990;163:1883-90
Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast (Rodway et al). 1990;163:1546-51
Central venous pressure
Ultrasonographic guidance for central venous access (Greenspoon) (Letter); (Lee) (Reply). 1990;163: 252
Cerclage; *see* **Pregnancy, prolonged**
Cerclage, cervical; *see* **Cervical incompetence**
Cerebral aneurysm
In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1
Cerebral circulation; *see* **Brain**
Cerebral palsy
Cerebral palsy and intrapartum fetal acidemia (Goodlin) (Letter); (Dennis et al) (Reply). 1990;163:266-7
Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33
Cerebral veins
In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1
Cerebrovascular disorders
Past use of oral contraceptives and cardiovascular disease: a meta-analysis in the context of the Nurses' Health Study (Stampfer et al). 1990;163:285-91 (Supplement)
Cervical cerclage; *see* **Cervical incompetence**
Cervical conization; *see* **Biopsy**
Cervical dilators; *see* **Surgical instruments**
Cervical incompetence
The Shirodkar operation: a reappraisal (Frieden et al). 1990;163:830-3
Cervical pregnancy; *see* **Pregnancy, ectopic**
Cervical ripening; *see* **Cervix uteri**; **Labor, induced**
Cervix mucus
Antisperm antibodies in women undergoing intrauterine insemination (Goldberg et al). 1990;163:65-8
Cervix neoplasms
Cervical cancer: Role for progesterone during pregnancy and contraception? (Stendahl and Rogo). 1990;163:685-6 (Letter)
Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies (Mutch et al). 1990;163:1920-4
Smoking and the antioxidant ascorbic acid: plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women (Basu et al). 1990;163:1948-52
Successful conservative management of primary nonmetastatic cervical choriocarcinoma (Ben-Chetrit et al). 1990;163:1161-3
Trend of cervical cancer in Jewish women (Baruch and Menczer) (Letter); (Glezerman et al) (Reply). 1990;163:703-4
The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes (Lewandowski et al). 1990;163:1333-7
Cervix uteri
Conservative management of cervical pregnancy with subsequent fertility (Ayromlooi) (Letter); (Bachus) (Reply). 1990;163:1093-5
Modified laparoscopy for monitoring transcervical surgery (Lin and Iwata). 1990;163:243-4 (Letter)
Morphologic study of changes of collagenous tissue in the amnion and cervix during prostaglandin-induced abortion and delivery (Manabe and Yoshida). 1990; 163:52-6
Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length (Andersen et al). 1990;163:859-67
Preventing cervical conization by achieving satisfactory col-

poscopy with hygroscopic cervical dilators (Stern et al). 1990;163:176-7
Role of hormones in cervical ripening (Sert). 1990;163:705-7 (Letter)

Cervix uteri, animal
Effect of intravaginal application of prostaglandin E_2 gel on the mechanical properties of the ovine cervix uteri at term (Owiny and Fitzpatrick). 1990;163:657-60

Cesarean section
Catastrophic complications of previous cesarean section (Chazotte and Cohen). 1990;163:738-42
Epidural analgesia and cesarean section for dystocia (Chestnut) (Letter); (Thorp and Parisi) (Reply). 1990;163:247-9
Extensive vulvar and vaginal varicella necessitating abdominal delivery (Sites et al). 1990;163:1630-1
Fetal gastroschisis and omphalocele: Is cesarean section the best mode of delivery? (Lewis et al). 1990;163:773-5
Fetal heart rate monitoring during cesarean section (Dunn) (Letter); (Petrikovsky et al) (Reply). 1990;163:253
The incidence of significant fetomaternal hemorrhage in patients undergoing cesarean section (Feldman et al). 1990;163:855-8
Labor epidural analgesia and dystocia-related cesarean section (Downing and Knape). 1990;163:1096-7 (Letter)
Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33
Reducing cesarean sections at a teaching hospital (Sanchez-Ramos et al). 1990;163:1081-8
Safe motherhood: Cesarean section or symphysiotomy? (van Roosmalen). 1990;163:1-4 (Clin. opinion)
Surgical staples in cesarean section: a randomized controlled trial (Villeneuve et al). 1990;163:1641-6
Ultrasonographic evaluation of uteroplacental blood flow patterns of abnormally located and adherent placentas (Guy et al). 1990;163:723-7
Vaginal birth after abdominal delivery (Thorp and Bowes). 1990;163:1101-2 (Letter)
Vaginal birth after cesarean section: an appraisal of the reappraisal (Flamm) (Letter); (Yetman) (Reply). 1990;163:691-2

Chemotherapy
Expression of P-glycoprotein in epithelial ovarian cancer: evaluation as a marker of multidrug resistance (Rubin et al). 1990;163:69-73

Chest wall; *see* **Thorax**

Chickenpox
Extensive vulvar and vaginal varicella necessitating abdominal delivery (Sites et al). 1990;163:1630-1

Chorioamnionitis
Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor (Romero et al). 1990;163:968-74
In vitro inhibition of esterase activity in amniotic fluid: comparison with bacterial cultures (Hoskins et al). 1990;163:1944-7
Perinatal survival with expectant management of midtrimester rupture of membranes (Major and Kitzmiller). 1990;163:838-44
Preterm delivery: a risk factor for retained placenta (Romero et al). 1990;163:823-5
Secretion of phospholipases by bacterial pathogens may initiate preterm labor (Bennett et al). 1990;163:241-2 (Letter)

Choriocarcinoma
Isolation and characterization of the gene from a human genome encoding 17β-estradiol dehydrogenase: a comparison of Jar and BeWo choriocarcinoma cell lines (Long et al). 1990;163:1976-81
Successful conservative management of primary nonmetastatic cervical choriocarcinoma (Ben-Chetrit et al). 1990;163:1161-3

Chorion
Cases with ruptured membranes that "reseal" (Johnson et al). 1990;163:1024-32
Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis (Chazotte et al). 1990;163:1180-1

Chorionic gonadotropins; *see* **Gonadotropins, chorionic**

Chorionic villi sampling
Antenatal diagnosis of molybdenum cofactor deficiency (George et al). 1990;163:1203-4
Detection of congenital toxoplasmosis by chorionic villus sampling and early amniocentesis (Foulon et al). 1990;163:1511-3
Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: an accurate and rapid method for detection of fetal chromosome abnormalities (Shulman et al). 1990;163:1606-9
An evaluation of the chorionic villus sampling learning curve (Silver et al). 1990;163:917-22
Postmortem chorionic villus sampling is a better method for cytogenetic evaluation of early fetal loss than culture of abortus material (Johnson et al). 1990;163:1505-10
Second-trimester placental biopsy for rapid fetal karyotyping (Cameron et al). 1990;163:931-4
Transcervical chorionic villus sampling and amniocentesis: a comparison of reliability, culture findings, and fetal outcome (Ferguson et al). 1990;163:926-31

Chromosome abnormalities
The association of single umbilical artery with cytogenetically abnormal pregnancies (Saller et al). 1990;163:922-5
Cytogenetic uncertainties surrounding the fragile X in Martin-Bell syndrome (Sindwani and Verma). 1990;163:1713-4 (Letter)
Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: an accurate and rapid method for detection of fetal chromosome abnormalities (Shulman et al). 1990;163:1606-9
Placental histology in fetuses between 18 and 23 weeks' gestation with abnormal karyotype (Kuhlmann et al). 1990;163:1264-70
Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21 (Ginsberg et al). 1990;163:1186-90

Chronic renal disease; *see* **Kidney failure, chronic**

Cigarette smoking; *see* **Smoking**

Circadian rhythm
Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle (Figueroa et al). 1990;163:648-54

Circumcision
Effectiveness of 1% lidocaine dorsal penile nerve block in infant circumcision (Arnett et al). 1990;163:1074-80

Cisplatin
The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemo-

Cisplatin—cont'd
therapy after surgical cytoreduction (Hunter et al). 1990;163:1164-7
Clindamycin
Bacterial vaginosis: diagnostic and pathogenetic findings during topical clindamycin therapy (Livengood et al). 1990;163:515-20
Clindamycin dosage scheduling for acute pelvic infection (Chatwani et al). 1990;163:240 (Letter)
Clinical opinion
Clinical opinion. 1990;163:1-8, 447-52, 1111-9, 1421-9
Clinical studies; *see* **Evaluation studies**
Clomiphene
Luteal insufficiency: correlation between endometrial dating and integrated progesterone output in clomiphene citrate–induced cycles (Hecht et al). 1990;163:1986-91
Clonidine
Effects of clonidine on breathing movements and electrocortical activity in the fetal lamb (Bamford et al). 1990;163:661-8
Clostridiopeptidase A
Changes in active and latent collagenase in human placenta around the time of parturition (Rajabi et al). 1990;163:499-503
Closure, surgical; *see* **Surgery, operative**
Cocaine
Cocaine effects on fetal behavioral state (Tabor and Soffici) (Letter); (Hume) (Reply). 1990;163:1364-5
Cocaine-related maternal death (Burkett et al). 1990;163:40-1
Lack of specific placental abnormality associated with cocaine use (Gilbert et al). 1990;163:998-9
Metabolism of cocaine by human placentas: implications for fetal exposure (Roe et al). 1990;163:715-8
The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening (Gillogley et al). 1990;163:1535-42
Prevalence of use of cocaine and other substances in an obstetric population (Matera et al). 1990;163:797-801
Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes (Plessinger and Woods). 1990;163:1659-64
The relationship of cocaine use to syphilis and human immunodeficiency virus infections among inner city parturient women (Minkoff et al). 1990;163:521-6
Rupture of unscarred uterus in primigravid woman in association with cocaine abuse (Gonsoulin et al). 1990;163:526-7
Coitus
Hemoperitoneum as a result of coital injury without associated vaginal injury (McColgin et al). 1990;163:1503-5
Collagen
Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81
Morphologic study of changes of collagenous tissue in the amnion and cervix during prostaglandin-induced abortion and delivery (Manabe and Yoshida). 1990;163:52-6
Collagenase; *see* **Clostridiopeptidase A**
Colonic neoplasms
Colon cancer in pregnancy with elevated maternal serum α-fetoprotein level at presentation (Gonsoulin et al). 1990;163:1172-3
Colony-stimulating factor
Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells (Dudley et al). 1990;163:1890-3
Color flow Doppler; *see* **Flowmeters**
Colposcopy
Colposcopy in diethylstilbestrol-exposed offspring (Gimbel) (Letter); (Helmerhorst) (Reply). 1990;163:703
Preventing cervical conization by achieving satisfactory colposcopy with hygroscopic cervical dilators (Stern et al). 1990;163:176-7
Combination chemotherapy; *see* **Drug therapy, combination**
Computed tomography; *see* **Tomography, x-ray computed**
Computer-assisted diagnosis; *see* **Diagnosis, computer-assisted**
Conception; *see* **Fertilization**
Congenital abnormalities; *see* **Abnormalities**
Congenital cytomegalovirus infection; *see* **Cytomegaloviruses**
Congenital infection; *see* **Infection, congenital**
Congenital toxoplasmosis; *see* **Toxoplasmosis, congenital**
Consensus development statement
Consensus development statement. 1990;163:1689-712
Consultants
Consultants for the American Journal of Obstetrics and Gynecology, September 1, 1989 — August 31, 1990. 1990;163:1771-80
Contraception
Cervical cancer: Role for progesterone during pregnancy and contraception? (Stendahl and Rogo). 1990;163:685-6 (Letter)
Contraception and ectopic pregnancy risk (Franks et al). 1990;163:1120-3
Contraception, immunologic
Lymphocyte proliferative response to fertilization antigen in patients with antisperm antibodies (Naz et al). 1990;163:610-3
Contraceptives, oral
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Clinical acceptability of monophasic gestodene (Düsterberg and Brill). 1990;163:1398-403 (Supplement)
Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: results of a controlled, randomized, prospective study (Kjos et al). 1990;163:1822-7
Effects of oral contraceptives on lipid metabolism (Gevers Leuven et al). 1990;163:1410-3 (Supplement)
Factors affecting the enterohepatic circulation of oral contraceptive steroids (Orme and Back). 1990;163:2146-52 (Symposium)
First European Conference on Sex Steroids and Metabolism: introductory remarks (Jespersen et al). 1990;163:273 (Supplement)
A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4
Formation, metabolism, and physiologic importance of catecholestrogens (Ball and Knuppen). 1990;163:2163-70 (Symposium)
Gastrointestinal disease and oral contraception (Hanker). 1990;163:2204-7 (Symposium)
Gastrointestinal metabolism of contraceptive steroids (Back et al). 1990;163:2138-45 (Symposium)
Influence of oral contraceptives on drug therapy (Teichmann). 1990;163:2208-13 (Symposium)

Inhibition of oral contraceptive steroid-metabolizing enzymes by steroids and drugs (Guengerich). 1990;163: 2159-63 (Symposium)
Inhibition of ovulation by low-dose monophasic contraceptive containing gestodene (Thomas and Vankrieken). 1990;163:1404-9 (Supplement)
Interactions with oral contraceptives (Fotherby). 1990;163:2153-9 (Symposium)
Oral contraception in disease states (Breckwoldt et al). 1990;163:2213-6 (Symposium)
Oral contraceptives, lipoproteins, and atherosclerosis (Adams et al). 1990;163:1388-92 (Supplement)
Oral contraceptives and breast cancer (Schlesselman). 1990;163:1379-87 (Supplement)
Oral contraceptives and myocardial infarction (Thorneycroft). 1990;163:1393-7 (Supplement)
Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism (Jung-Hoffmann and Kuhl). 1990;163:2183-97 (Symposium)
Pharmacokinetics of ethinyl estradiol and mestranol (Goldzieher and Brody). 1990;163:2114-9 (Symposium)
Pharmacokinetics of gestagens: some problems (Fotherby). 1990;163:323-8 (Supplement)
Pharmacokinetics of gestodene and ethinyl estradiol after oral administration of a monophasic contraceptive (Täuber et al). 1990;163:1414-20 (Supplement)
Pharmacokinetics of oral contraceptive steroids and drug interaction: conclusion (Kuhl). 1990;163:2216-8 (Symposium)
Pharmacokinetics of oral contraceptive steroids and drug interaction: introduction (Kuhl). 1990;163:2113 (Symposium)
Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women (Kuhnz). 1990;163:2120-7 (Symposium)
Pharmacologic and pharmacokinetic characteristics of norgestimate and its metabolites (McGuire et al). 1990;163:2127-31 (Symposium)
Prodrugs: Advantage or disadvantage? (Hammerstein). 1990;163:2198-203 (Symposium)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Selected aspects of the pharmacokinetics and metabolism of ethinyl estrogens and their clinical implications (Goldzieher). 1990;163:318-22 (Supplement)
Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin (Bergink et al). 1990;163:2132-7 (Symposium)

Contraceptives, oral, adverse effects

Cardiovascular death among women under 40 years of age using low-estrogen oral contraceptives and intrauterine devices in Finland from 1975 to 1984 (Hirvonen and Idänpään-Heikkilä). 1990;163:281-4 (Supplement)
Clinical aspects of the relationship between oral contraceptives, abnormalities in carbohydrate metabolism, and the development of cardiovascular disease (Gaspard and Lefebvre). 1990;163:334-43 (Supplement)
Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease (Kelleher). 1990;163:392-5 (Supplement)
The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction (Hoppe). 1990;163:388-91 (Supplement)
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial (Jandrain et al). 1990;163:378-81 (Supplement)
Effects of long-term gestodene-containing oral contraceptive administration on hemostasis (Abbate et al). 1990;163:424-9 (Supplement)
Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid (Jespersen et al). 1990;163:396-403 (Supplement)
Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom Study (Simon et al). 1990;163:382-7 (Supplement)
Effects of three combined oral contraceptive preparations containing desogestrel plus ethinyl estradiol on lipid metabolism in comparison with two levonorgestrel preparations (Kloosterboer and Rekers). 1990;163:370-3 (Supplement)
Enhanced generation and resolution of fibrin in women above the age of 30 years using oral contraceptives low in estrogen (Gram et al). 1990;163:438-42 (Supplement)
An epidemiologic survey of cardiovascular disease in women taking oral contraceptives (Thorogood and Vessey). 1990;163:274-81 (Supplement)
Estrogenic effect of gestodene- or desogestrel-containing oral contraceptives on lipoprotein metabolism (Gevers Leuven et al). 1990;163:358-62 (Supplement)
Hemostasis profile in women taking low-dose oral contraceptives (David et al). 1990;163:420-3 (Supplement)
Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system (Beck-Nielsen et al). 1990;163:292-5 (Supplement)
Influence of oral contraceptives on coagulation tests in native blood and plasma (Kunz et al). 1990;163:417-20 (Supplement)
Low-dose oral contraceptives and carbohydrate metabolism (Godsland et al). 1990;163:348-53 (Supplement)
Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level (Skouby et al). 1990;163:343-8 (Supplement)
Metabolic changes during the normal menstrual cycle: a longitudinal study (Lebech et al). 1990;163:414-6 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Oral contraceptives in risk groups: Exclusion or monitoring? (Guillebaud). 1990;163:443-6 (Supplement)
Past use of oral contraceptives and cardiovascular disease: a meta-analysis in the context of the Nurses' Health Study (Stampfer et al). 1990;163:285-91 (Supplement)
Role of plasma lipoproteins in the pathogenesis of atherosclerotic disease, with special reference to sex hormone effects (Tikkanen). 1990;163:296-304 (Supplement)

Contraceptives, oral, adverse effects—cont'd
Some aspects of the relationship between oral contraceptives, lipid abnormalities, and cardiovascular disease (Samsioe and Mattsson). 1990;163:354-8 (Supplement)
Studies on the mechanism of action of oral contraceptives with regard to fibrinolytic variables (Kooistra et al). 1990;163:404-13 (Supplement)
Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives (Kuhl et al). 1990;163:363-9 (Supplement)
Why do we need pharmacokinetic studies? (Hvidberg). 1990;163:316-8 (Supplement)
Cordocentesis; *see* **Fetal blood**
Coronary disease
An epidemiologic survey of cardiovascular disease in women taking oral contraceptives (Thorogood and Vessey). 1990;163:274-81 (Supplement)
Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women (Wild et al). 1990;163:1998-2005
Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system (Beck-Nielsen et al). 1990;163:292-5 (Supplement)
Low-dose oral contraceptives and carbohydrate metabolism (Godsland et al). 1990;163:348-53 (Supplement)
Oral contraception in disease states (Breckwoldt et al). 1990;163:2213-6 (Symposium)
Prevalence and determinants of estrogen replacement therapy in elderly women (Cauley et al). 1990;163:1438-44
Coronary vessels, physiopathology
Postpartum coronary artery dissection followed by heart transplantation (Curiel et al). 1990;163:538-9
Corpus luteum
Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels (Kratzer and Taylor). 1990;163:1497-502
Oxytocin and vasopressin binding sites in human and bovine ovaries (Fuchs et al). 1990;163:1961-7
Corrections
The American Board of Obstetrics and Gynecology, Inc. (1990;162:1645-68). 1990;163:2030
Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population (Owen et al) (1990;163:873-9). 1990;163:2030
Fetal thoracic circumference: a prospective cross-sectional study with real-time ultrasound (Fong et al) (1988;158:1154-60). 1990;163:1363
Transabdominal and transvaginal ultrasonography in the diagnosis of ectopic pregnancy: a comparative study (1990;163:123-9). 1990;163:2030
Treatment-dependent and treatment-independent pregnancy among women with periadnexal adhesions (Tulandi et al) (1990;162:354-7). 1990;163:271
Correspondence
Letters. 1990;163:240-71, 677-712, 1089-107, 1364-77, 1713-7, 2024-30
Corticosteroid binding-globulin; *see* **Transcortin**
Corticosteroids; *see* **Adrenal cortex hormones**
Corticotropin-releasing hormone
Corticotropin-releasing hormone in amniotic fluid (Laatikainen) (Letter); (Sasaki) (Reply). 1990;163:1102-3
Mechanism of action of interleukin-1β in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells (Petraglia et al). 1990;163:1307-12
Cortisol; *see* **Hydrocortisone**
Cost benefit analysis
Cost-effectiveness of intrapartum screening and treatment for maternal group B streptococci colonization (Strickland et al). 1990;163:4-8 (Clin. opinion)
Cost effectiveness; *see* **Cost benefit analysis**
Crack cocaine; *see* **Cocaine**
Curettage
Experience with the EndoPap device for the cytologic detection of uterine cancer and its precursors: a comparison of the EndoPap with fractional curettage or hysterectomy (LaPolla et al). 1990;163:1055-60
Current development
Current development. 1990;163:162-9, 1282-93
Cushing's syndrome
Ketoconazole treatment of Cushing's syndrome in pregnancy (Divers). 1990;163:1101 (Letter)
Cyclic AMP; *see* **Adenosine cyclic monophosphate**
Cyclooxygenase; *see* **Prostaglandin synthase**
Cynomolgus monkeys; *see* ***Macaca fascicularis***
Cyproterone
Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial (Jandrain et al). 1990;163:378-81 (Supplement)
Cystadenoma
Hemoperitoneum as a result of coital injury without associated vaginal injury (McColgin et al). 1990;163:1503-5
Papillary cystadenoma of the broad ligament in von Hippel-Lindau disease (Korn et al). 1990;163:596-8
Cystic angiomatosis; *see* **Angiomatosis**
Cystic hygroma; *see* **Lymphangioma**
Cysts
Endometrioma of the liver (Rovati et al). 1990;163:1490-2
Management of selected cystic adnexal masses in postmenopausal women by operative laparoscopy: a pilot study (Parker and Berek). 1990;163:1574-7
Cytochrome P-450
Inhibition of oral contraceptive steroid-metabolizing enzymes by steroids and drugs (Guengerich). 1990;163:2159-63 (Symposium)
Cytogenetics
Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: an accurate and rapid method for detection of fetal chromosome abnormalities (Shulman et al). 1990;163:1606-9
Cytomegalic inclusion disease, diagnosis
Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation from amniotic fluid (Weiner and Grose). 1990;163:1253-5
Cytomegaloviruses
Prenatal diagnosis of congenital cytomegalovirus infection: two decades later (Grose and Weiner). 1990;163:447-50 (Clin. opinion)
Prenatal diagnosis of fetal intracranial calcifications (Koga et al). 1990;163:1543-5
Cytotoxicity, immunologic
Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions. III. Cytotoxic potential in vitro and characterization of antigen(s) (Kutteh et al). 1990;163:1301-6
Cytotoxicity tests, immunologic
A comparison of sperm antibody assays (Daru and Mathur). 1990;163:1622-9

Cytotrophoblastic cell analysis; *see* **Cytogenetics**

D

Danazol
Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis (Sakata et al). 1990;163:1679-84
Death, maternal; *see* **Maternal mortality**
Deceleration
The effect of amnioinfusion on uterine pressure and activity: a preliminary report (Posner et al). 1990;163:813-8
Effect of uterine contractility and maternal hypotension on prolonged decelerations after bupivacaine epidural anesthesia (Steiger and Nageotte). 1990;163:808-12
Decidua
Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta (Cheung et al). 1990;163:1445-9
Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells (Dudley et al). 1990;163:1890-3
Dehydroepiandrosterone
Role of hormones in cervical ripening (Sert). 1990;163:705-7 (Letter)
Delivery
A comparison of the effect of epidural, general, and no anesthesia on funic acid-base values by stage of labor and type of delivery (Shyken et al). 1990;163:802-7
The effect of mode of delivery on the perinatal outcome in fetuses with abdominal wall defects (Moretti et al). 1990;163:833-8
Fetal gastroschisis and omphalocele: Is cesarean section the best mode of delivery? (Lewis et al). 1990;163:773-5
Is indicated or spontaneous preterm delivery more advantageous for the fetus? (Owen et al). 1990;163:868-72
Preterm delivery: a risk factor for retained placenta (Romero et al). 1990;163:823-5
Vaginal birth after abdominal delivery (Thorp and Bowes). 1990;163:1101-2 (Letter)
Vaginal birth after cesarean section: an appraisal of the reappraisal (Flamm) (Letter); (Yetman) (Reply). 1990;163:691-2
Vaginal breech delivery: a five-year prospective evaluation of a protocol using computed tomographic pelvimetry (Christian et al). 1990;163:848-55
Deoxyribonucleic acid fingerprints; *see* **Nucleotide mapping**
Descending aorta; *see* **Aorta, thoracic**
Desogestrel
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Gastrointestinal metabolism of contraceptive steroids (Back et al). 1990;163:2138-45 (Symposium)
Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism (Jung-Hoffmann and Kuhl). 1990;163:2183-97 (Symposium)
Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin (Bergink et al). 1990;163:2132-7 (Symposium)
Desogestrel/ethinyl estradiol
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial (Jandrain et al). 1990;163:378-81 (Supplement)
Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid (Jespersen et al). 1990;163:396-403 (Supplement)
Effects of three combined oral contraceptive preparations containing desogestrel plus ethinyl estradiol on lipid metabolism in comparison with two levonorgestrel preparations (Kloosterboer and Rekers). 1990;163:370-3 (Supplement)
Estrogenic effect of gestodene- or desogestrel-containing oral contraceptives on lipoprotein metabolism (Gevers Leuven et al). 1990;163:358-62 (Supplement)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives (Kuhl et al). 1990;163:363-9 (Supplement)
Dextrans
Safety of 32% dextran 70 for hysteroscopy (Ljungström) (Letter); (Jedeikin and Olsfanger) (Reply). 1990;163:2029-30
Diabetes, pregnancy and; *see* **Pregnancy in diabetes**
Diabetes insipidus
Indomethacin treatment for polyhydramnios and subsequent infantile nephrogenic diabetes insipidus (Smith et al). 1990;163:98-9
Diabetes mellitus
Infants of diabetic mothers with accelerated fetal growth by ultrasonography: Are they all alike? (Keller et al). 1990;163:893-7
Oral contraception in disease states (Breckwoldt et al). 1990;163:2213-6 (Symposium)
Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests (Kjos et al). 1990;163:898-903
Diabetes mellitus, insulin-dependent
Diabetic ketoacidosis and insulin resistance with subcutaneous terbutaline infusion: a case report (Tibaldi et al). 1990;163:509-10
Diabetes mellitus, non-insulin-dependent
Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months post partum (Kjos et al). 1990;163:93-8
Diabetic ketoacidosis; *see* **Acidosis, diabetic; Ketosis, diabetic**
Diabetic mothers; *see* **Pregnancy in diabetes**
Diagnosis, computer-assisted
Blood pressure measured telemetrically from home throughout pregnancy (Mooney et al). 1990;163:30-6
Ultrasonographic fetal weight estimation by an integrated computer-assisted system: Can each laboratory improve its accuracy? (Thompson et al). 1990;163:986-95
Vaginal breech delivery: a five-year prospective evaluation

Diagnosis, computer-assisted—cont'd
of a protocol using computed tomographic pelvimetry (Christian et al). 1990;163:848-55
Diagnosis, differential
Use of serial human chorionic gonadotropin measurements to differentiate between intrauterine and ectopic pregnancies (Kadar) (Letter); (Lindblom et al) (Reply). 1990;163:259-61
Diagnosis, laboratory
Disorders of the hemostatic system and the risk of the development of thrombotic and cardiovascular diseases: limitations of laboratory diagnosis (Kluft). 1990;163:305-12 (Supplement)
The importance of considering clinical usefulness in the evaluation of medical laboratory tests (Sever). 1990;163:678-9 (Letter)
Laboratory identification of the lupus anticoagulant in normal pregnancy and pregnancy-induced hypertension (Riveros et al) (Letter); (Lockwood) (Reply). 1990;163:704-5
Diazoxide
Suppression of serum insulin level by diazoxide does not alter serum testosterone of sex hormone–binding globulin levels in healthy, nonobese women (Nestler et al). 1990;163:1243-6
Diethylstilbestrol, adverse effects
Colposcopy in diethylstilbestrol-exposed offspring (Gimbel) (Letter); (Helmerhorst) (Reply). 1990;163:703
Digoxin-like immunoreactive factor
Digoxin-like immunoreactive factor and respiratory distress syndrome (Goodlin). 1990;163:1091 (Letter)
Dilapan; *see* **Surgical instruments**
Dinoprost
Conservative treatment of ovarian pregnancy by local prostaglandin $F_{2\alpha}$ injection (Koike et al). 1990;163:696 (Letter)
Critical comments on the treatment of tubal pregnancy with prostaglandins (Dietl and Lippert) (Letter); (Egarter and Husslein) (Reply). 1990;163:707-8
Double-blind comparison of intravaginal prostaglandin E_2 gel and "chip" for preinduction cervical ripening (Smith et al). 1990;163:845-7
Dinoprostone
Effect of intravaginal application of prostaglandin E_2 gel on the mechanical properties of the ovine cervix uteri at term (Owiny and Fitzpatrick). 1990;163:657-60
Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells (Kniss et al). 1990;163:1883-90
The role of fetal membranes in regulating production, transport, and metabolism of prostaglandin E_2 during labor (McCoshen et al). 1990;163:1632-40
Uterine hyperstimulation after low-dose prostaglandin E_2 therapy: tocolytic treatment in 181 cases (Egarter et al). 1990;163:794-6
Disease models, animal
Ovarian granulosa cell tumorigenesis in SWR-derived F1 hybrid mice: preneoplastic follicular abnormality and malignant disease progression (Tennent et al). 1990;163:625-34
Disseminated intravascular coagulation
Explanation of mechanism of Hyskon solution reaction needed (Loffer). 1990;163:2029 (Letter)
DNA, viral
Carbon dioxide laser energy disperses human papillomavirus deoxyribonucleic acid onto treatment fields (Ferenczy et al). 1990;163:1271-4
DNA fingerprinting; *see* **Nucleotide mapping**
DNA probes
Differential diagnosis between complete mole and hydropic abortus by deoxyribonucleic acid fingerprints (Nobunaga et al). 1990;163:634-8
DNA probes, HPV
The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes (Lewandowski et al). 1990;163:1333-7
Donor oocytes; *see* **Oocytes**
Donors; *see* **Tissue donors**
Dopamine
Hypothalamic amenorrhea: clinical perspectives, pathophysiology, and management (Liu). 1990;163:1732-6 (Supplement)
Doppler echocardiography; *see* **Echocardiography, Doppler**
Doppler velocimetry; *see* **Flowmeters**
Down's syndrome
Maternal serum Down syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin (Macri et al). 1990;163:1248-53
Maternal serum unconjugated estriol levels are lower in the presence of fetal Down syndrome (Haddow et al) (Letter); (Macri et al) (Reply). 1990;163:1372-4
Potential prenatal predictions of Down syndrome: a statistical analysis (O'Brien et al). 1990;163:1796-8
Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femur length ratio (Marquette et al). 1990;163:1604-5
Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21 (Ginsberg et al). 1990;163:1186-90
Drug abuse; *see* **Substance abuse**
Drug interactions
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Factors affecting the enterohepatic circulation of oral contraceptive steroids (Orme and Back). 1990;163:2146-52 (Symposium)
Formation, metabolism, and physiologic importance of catecholestrogens (Ball and Knuppen). 1990;163:2163-70 (Symposium)
Gastrointestinal disease and oral contraception (Hanker). 1990;163:2204-7 (Symposium)
Gastrointestinal metabolism of contraceptive steroids (Back et al). 1990;163:2138-45 (Symposium)
Influence of oral contraceptives on drug therapy (Teichmann). 1990;163:2208-13 (Symposium)
Inhibition of oral contraceptive steroid-metabolizing enzymes by steroids and drugs (Guengerich). 1990;163:2159-63 (Symposium)
Interactions with oral contraceptives (Fotherby). 1990;163:2153-9 (Symposium)
Oral contraception in disease states (Breckwoldt et al). 1990;163:2213-6 (Symposium)
Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism (Jung-Hoffmann and Kuhl). 1990;163:2183-97 (Symposium)
Pharmacokinetics of ethinyl estradiol and mestranol (Goldzieher and Brody). 1990;163:2114-9 (Symposium)
Pharmacokinetics of oral contraceptive steroids and drug

interaction: conclusion (Kuhl). 1990;163:2216-8 (Symposium)
Pharmacokinetics of oral contraceptive steroids and drug interaction: introduction (Kuhl). 1990;163:2113 (Symposium)
Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women (Kuhnz). 1990;163:2120-7 (Symposium)
Pharmacologic and pharmacokinetic characteristics of norgestimate and its metabolites (McGuire et al). 1990;163:2127-31 (Symposium)
Prodrugs: Advantage or disadvantage? (Hammerstein). 1990;163:2198-203 (Symposium)
Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin (Bergink et al). 1990;163:2132-7 (Symposium)

Drug resistance
Expression of P-glycoprotein in epithelial ovarian cancer: evaluation as a marker of multidrug resistance (Rubin et al). 1990;163:69-73

Drug therapy
The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemotherapy after surgical cytoreduction (Hunter et al). 1990;163:1164-7
Successful conservative management of primary nonmetastatic cervical choriocarcinoma (Ben-Chetrit et al). 1990;163:1161-3

Drug therapy, combination
Combination therapy has potential complications (Frohlich) (Letter); (Snyder and Cardwell) (Reply). 1990;163:244

Ductus arteriosus
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66

Dyspareunia
Laparoscopic ventrosuspensions (Yoong). 1990;163:1151-3

Dystocia
Can shoulder dystocia be predicted? (Gimbel) (Letter); (O'Leary) (Reply). 1990;163:680
Can shoulder dystocia be prevented? (Nagey) (Letter); (O'Leary) (Reply). 1990;163:1095-6
Epidural analgesia and cesarean section for dystocia (Chestnut) (Letter); (Thorp and Parisi) (Reply). 1990;163:247-9
Labor epidural analgesia and dystocia-related cesarean section (Downing and Knape). 1990;163:1096-7 (Letter)

E

Eating disorders
Infertility and eating disorders (Stewart et al). 1990;163:1196-9

Echo-planar imaging; *see* **Magnetic resonance imaging**

Echocardiography
Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus (Carlson et al). 1990;163:1785-7

Echocardiography, Doppler
Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry (Ikeda and Mori). 1990;163:494-8
Blood flow velocity and pulsatile diameter changes in the fetal descending aorta: a longitudinal study (Stale et al). 1990;163:26-9
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66
Prenatal diagnosis of a chest wall mass with ultrasonography and Doppler velocimetry: a case report (Smith et al). 1990;163:567-9
Relationship between the ovine fetal umbilical artery blood flow waveform and umbilical vascular resistance (Irion and Clark). 1990;163:222-9

Eclampsia
Eclampsia. VI. Maternal-perinatal outcome in 254 consecutive cases (Sibai). 1990;163:1049-55
Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986 (Saftlas et al). 1990;163:460-5
Magnesium sulfate is not an ideal anticonvulsant (Goodlin) (Letter); (Sibai) (Reply). 1990;163:1714-5
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. 1990;163:1691-712 (Supplement)
Phenytoin and magnesium sulfate in eclampsia (Dommisse) (Letter); (Ryan) (Reply). 1990;163:1089-91

Ectopic pregnancy; *see* **Pregnancy, ectopic**

Edema
False-negative syphilis screening: the prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy (Berkowitz et al). 1990;163:975-7

Editorial
Editorial. 1990;163:1109-10

Education, medical
Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? (Chervenak and McCullough). 1990;163:1425-9 (Clin. opinion)

Egg transport; *see* **Ovum transport**

Eicosanoids
Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome (Brown et al). 1990;163:1853-61

Electric conductivity
Ovulation prediction from cyclic changes in salivary electrical conductivity (Loewit et al). 1990;163:708-10 (Letter)

Electrocardiography
Effects of ritodrine tocolysis on cardiac isozymes and electrocardiography (Hendricks and Katz) (Letter); (Hadi and Albazzaz) (Reply). 1990;163:699-701

ELISA; *see* **Enzyme-linked immunosorbent assay**

Embolism, air
Hyperbaric oxygen therapy for air embolism complicating operative hysteroscopy (Tur-Kaspa) (Letter); (Baggish) (Reply). 1990;163:680-1
Sapphire probes in obstetric laser surgery (Corson) (Letter); (Baggish and Daniell) (Reply). 1990;163:246-7

Embolism, amniotic fluid
Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy (Esposito et al). 1990;163:572-4

Embryo
Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm (Hardardottir et al). 1990;163:1925-31
Embryo migration responsible for ectopic pregnancies (Rizk). 1990;163:1369 (Letter)
Embryopathic effects of short-term exposure to hypoglycemia in mouse embryos in vitro (Smoak and Sadler). 1990;163:619-24

Embryo development; *see* **Fetal development**
Embryo implantation; *see* **Ovum implantation**
Endocrine diseases
Multiple endocrine adenomatosis type I in pregnancy (Smythe et al). 1990;163:1037-9
Endometrial carcinoma; *see* **Carcinoma**
Endometrioma of liver; *see* **Endometriosis**
Endometriosis
Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81
Endometrioma of the liver (Rovati et al). 1990;163:1490-2
Immunohistochemical localization of prostaglandin H synthase in the female reproductive tract and endometriosis (Van Voorhis et al). 1990;163:57-62
Studies of the spontaneous motility and the effect of histamine on isolated myometrial strips of the nonpregnant human uterus: the influence of various uterine abnormalities (Martinez-Mir et al). 1990;163:189-95
The use of serial CA 125 concentrations to monitor endometriosis in infertile women (Pittaway). 1990;163:1032-7
Endometriosis, animal
Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis (Sakata et al). 1990;163:1679-84
Endometrium
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163:1932-8
Endometrial assessment by vaginal ultrasonography before endometrial sampling in patients with postmenopausal bleeding (Goldstein et al). 1990;163:119-23
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84
Experience with the EndoPap device for the cytologic detection of uterine cancer and its precursors: a comparison of the EndoPap with fractional curettage or hysterectomy (LaPolla et al). 1990;163:1055-60
Immunohistochemical localization of prostaglandin H synthase in the female reproductive tract and endometriosis (Van Voorhis et al). 1990;163:57-62
Interleukin-1β, human leukocyte antigen DR1, and transforming growth factor-β expression in endometrium, placenta, and placental membranes (Kauma et al). 1990;163:1430-7
Endopap device; *see* **Uterus**
Endorphins
Hypothalamic amenorrhea: clinical perspectives, pathophysiology, and management (Liu). 1990;163:1732-6 (Supplement)
Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women (Jaffe et al). 1990;163:1727-31 (Supplement)
Endothelial cells; *see* **Endothelium, vascular**
Endothelium, vascular
Studies on the mechanism of action of oral contraceptives with regard to fibrinolytic variables (Kooistra et al). 1990;163:404-13 (Supplement)
Endothelium, vascular, animal
Role of the endothelium in adrenergic responses of mesenteric artery rings of pregnant rats (Parent et al). 1990;163:229-34
Enterohepatic circulation
Factors affecting the enterohepatic circulation of oral contraceptive steroids (Orme and Back). 1990;163:2146-52 (Symposium)
Enzyme-linked immunosorbent assay
Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients (Towers et al). 1990;163:965-7
Epidermal growth factor-urogastrone
Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells (Kniss et al). 1990;163:1883-90
Epidural analgesia; *see* **Analgesia, epidural**
Epithelium
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84
Epitopes; *see* **Antigenic determinants**
Erythroblastosis, fetal
Postinjection kinetics of antepartum Rh immune globulin (Witter et al). 1990;163:784-6
Erythrocytes, immunology
Acute fetal hemodynamic alterations after intrauterine transfusion for treatment of severe red cell alloimmunization (Moise et al). 1990;163:776-84
Erythromycin
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91
Esterases
In vitro inhibition of esterase activity in amniotic fluid: comparison with bacterial cultures (Hoskins et al). 1990;163:1944-7
Estradiol
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163:1932-8
Isolation and characterization of the gene from a human genome encoding 17β-estradiol dehydrogenase: a comparison of Jar and BeWo choriocarcinoma cell lines (Long et al). 1990;163:1976-81
The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants (Studd et al). 1990;163:1474-9
Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women (Cassidenti et al). 1990;163:1953-60
Estriol
Maternal serum unconjugated estriol levels are lower in the presence of fetal Down syndrome (Haddow et al) (Letter); (Macri et al) (Reply). 1990;163:1372-4
Estrogen antagonists
The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction (Hoppe). 1990;163:388-91 (Supplement)
Estrogen receptors, animal; *see* **Receptors, estrogen, animal**
Estrogen replacement therapy
Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys (Jayo et al). 1990;163:614-8

Prevalence and determinants of estrogen replacement therapy in elderly women (Cauley et al). 1990;163: 1438-44
The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants (Studd et al). 1990;163:1474-9
Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women (Cassidenti et al). 1990;163:1953-60

Estrogens
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial (Jandrain et al). 1990;163:378-81 (Supplement)
Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid (Jespersen et al). 1990;163:396-403 (Supplement)
Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom Study (Simon et al). 1990;163:382-7 (Supplement)
Efficacy and safety considerations in women with uterine leiomyomas treated with gonadotropin-releasing hormone agonists: the estrogen threshold hypothesis (Friedman et al). 1990;163:1114-9 (Clin. opinion)
Isolation and characterization of the gene from a human genome encoding 17β-estradiol dehydrogenase: a comparison of Jar and BeWo choriocarcinoma cell lines (Long et al). 1990;163:1976-81
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Prodrugs: Advantage or disadvantage? (Hammerstein). 1990;163:2198-203 (Symposium)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Selected aspects of the pharmacokinetics and metabolism of ethinyl estrogens and their clinical implications (Goldzieher). 1990;163:318-22 (Supplement)
Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives (Kuhl et al). 1990;163:363-9 (Supplement)

Ethics
Anencephalics as organ donors (Van Assche). 1990;163:599-600
Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? (Chervenak and McCullough). 1990;163:1425-9 (Clin. opinion)
Drug prophylaxis for human immunodeficiency virus–infected pregnant women: ethical considerations (Minkoff and Moreno). 1990;163:1111-4 (Clin. opinion)
Ethics and routine ultrasonography in pregnancy (Ewigman et al) (Letter); (Chervenak et al) (Reply). 1990;163:256-8
Ethics of new reproductive technologies (Blaskiewicz) (Letter); (Macnaughton) (Reply). 1990;163:1376

Ethinyl estradiol
Clinical acceptability of monophasic gestodene (Düsterberg and Brill). 1990;163:1398-403 (Supplement)
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial (Jandrain et al). 1990;163:378-81 (Supplement)
Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid (Jespersen et al). 1990;163:396-403 (Supplement)
Effects of oral contraceptives on lipid metabolism (Gevers Leuven et al). 1990;163:1410-3 (Supplement)
Factors affecting the enterohepatic circulation of oral contraceptive steroids (Orme and Back). 1990;163:2146-52 (Symposium)
Gastrointestinal metabolism of contraceptive steroids (Back et al). 1990;163:2138-45 (Symposium)
Inhibition of oral contraceptive steroid-metabolizing enzymes by steroids and drugs (Guengerich). 1990;163: 2159-63 (Symposium)
Inhibition of ovulation by low-dose monophasic contraceptive containing gestodene (Thomas and Vankrieken). 1990;163:1404-9 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism (Jung-Hoffmann and Kuhl). 1990;163:2183-97 (Symposium)
Pharmacokinetics of ethinyl estradiol and mestranol (Goldzieher and Brody). 1990;163:2114-9 (Symposium)
Pharmacokinetics of gestodene and ethinyl estradiol after oral administration of a monophasic contraceptive (Täuber et al). 1990;163:1414-20 (Supplement)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Selected aspects of the pharmacokinetics and metabolism of ethinyl estrogens and their clinical implications (Goldzieher). 1990;163:318-22 (Supplement)
Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives (Kuhl et al). 1990;163:363-9 (Supplement)

Ethinyl estradiol with desogestrel; *see* **Desogestrel/ethinyl estradiol**

Ethinyl estradiol with gestodene; *see* **Gestodene/ethinyl estradiol**

Ethnic groups
Glucose tolerance in pregnancy: ethnic variation and influence of body habitus (Green et al). 1990;163:86-92

Evaluation studies
The importance of considering clinical usefulness in the evaluation of medical laboratory tests (Sever). 1990;163:678-9 (Letter)

Exercise
The course of labor after endurance exercise during pregnancy (Clapp). 1990;163:1799-805
The effect of physical activity during pregnancy on preterm delivery and birth weight (Klebanoff et al). 1990;163:1450-6
Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive value for pregnancy-induced hypertension (Hume et al). 1990;163:826-9
Maternal exercise and early pregnancy outcome (Suzuki and Miller). 1990;163:1103 (Letter)
Neonatal morphometrics after endurance exercise during pregnancy (Clapp and Capeless). 1990;163:1805-11
Extrahepatic biliary tree; *see* **Biliary tract**
Eye, physiopathology
Neuroophthalmologic effects of intravenous magnesium sulfate (Digre et al). 1990;163:1848-52
Eye movements
Fetal eye movement assessed with real-time ultrasonography: Are there rapid and slow eye movements? (Horimoto et al). 1990;163:1480-4

F

Factor analysis, statistical
Potential prenatal predictions of Down syndrome: a statistical analysis (O'Brien et al). 1990;163:1796-8
Factor VII
Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease (Kelleher). 1990;163:392-5 (Supplement)
Effects of long-term gestodene-containing oral contraceptive administration on hemostasis (Abbate et al). 1990;163:424-9 (Supplement)
Fallopian tubes
The complex nature of egg transport through the oviduct (Egarter) (Letter); (Pulkkinen and Jaakkola) (Reply). 1990;163:687-8
Necrotizing fasciitis of the total abdominal wall after sterilization by partial salpingectomy (Cederna et al). 1990;163:138-9
False negative reactions
False-negative syphilis screening: the prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy (Berkowitz et al). 1990;163:975-7
Family planning
Contraception and ectopic pregnancy risk (Franks et al). 1990;163:1120-3
Fasciitis
Necrotizing fasciitis of the total abdominal wall after sterilization by partial salpingectomy (Cederna et al). 1990;163:138-9
Fatty liver
Pregnancy-induced hypertension and acute fatty liver of pregnancy: atypical presentations (Brown et al). 1990;163:1154-6
Recurrent acute fatty liver of pregnancy (Barton et al). 1990;163:534-8
Femovan; *see* **Gestodene/ethinyl estradiol**
Fertilization
5-Fluorouracil exposure during the period of conception: report on two cases (Odom et al). 1990;163:76-7
Lymphocyte proliferative response to fertilization antigen in patients with antisperm antibodies (Naz et al). 1990;163:610-3
Fertilization in vitro
Factors affecting embryo implantation after human in vitro fertilization: a hypothesis (Paulson et al). 1990;163:2020-3
Heterotopic pregnancy associated with assisted reproductive technology (Dimitry et al). 1990;163:244-5 (Letter)
How sperm commence movement and their isolation for in vitro fertilization and sex selection (Shettles). 1990;163:270 (Letter)
Tubal embryo successfully transferred in utero (Shettles). 1990;163:2026-7 (Letter)
Fertilization in vitro, animal
In vitro effect of RU 486 on sperm-egg interaction in mice (Juneja and Dodson). 1990;163:216-21
Platelet activating factor enhances in vitro fertilization of rabbit oocytes (Roudebush et al). 1990;163:1670-3
Fetal acidemia; *see* **Fetal distress**
Fetal acoustic stimulation; *see* **Acoustic stimulation**
Fetal anoxia
The association of intrapartum asphyxia in the mature fetus with newborn behavior (Low et al). 1990;163:1131-5
Definition of fetal asphyxia (Clark) (Letter); (Johnson and Richards) (Reply). 1990;163:1364
Fetal anoxia, animal
Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1990;163:1313-20
Role of vasopressin in mediation of fetal cardiovascular responses to acute hypoxia (Piacquadio et al). 1990;163:1294-300
Fetal blood
Continuing controversy in alloimmune thrombocytopenia: fetal hyperimmunoglobulinemia fails to prevent thrombocytopenia (Nicolini et al). 1990;163:1144-6
Cordocentesis and immune thrombocytopenia—continued (Weiner). 1990;163:1371-2 (Letter)
Detection of fetal blood contamination by amniotic fluid obtained during cordocentesis (Lazebnik et al). 1990;163:78-80
Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy (Skajaa et al). 1990;163:1323-33
Possible therapeutic applications of cordocentesis (Sherer and Abramowicz). 1990;163:1366-7 (Letter)
Relationship of fetal biophysical profile and blood gas values at cordocentesis in severely growth-retarded fetuses (Ribbert et al). 1990;163:569-71
Fetal blood, animal
Relationship between the ovine fetal umbilical artery blood flow waveform and umbilical vascular resistance (Irion and Clark). 1990;163:222-9
Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb (Paulick et al). 1990;163:1357-63
Fetal blood transfusion; *see* **Blood transfusion, intrauterine**
Fetal breathing movement; *see* **Fetal monitoring**
Fetal death
The association of maternal floor infarction of the placenta with adverse perinatal outcome (Andres et al). 1990;163:935-8
The effect of race on the relationship between fetal death

and altered fetal growth (Ferguson and Myers). 1990;163:1222-30
Induction of labor with mifepristone (RU 486) in intrauterine fetal death (Cabrol et al). 1990;163:540-2
Postmortem chorionic villus sampling is a better method for cytogenetic evaluation of early fetal loss than culture of abortus material (Johnson et al). 1990;163:1505-10
Recurrent adverse pregnancy outcome and antiphospholipid antibodies (Reece et al). 1990;163:162-9 (Curr. dev.)
Study results vary in count-to-10 method of fetal movement screening (Elbourne and Grant) (Letter); (Moore and Piacquadio) (Reply). 1990;163:264-5
Survival in a fetus with sacrococcygeal teratoma and hydrops (Nakayama). 1990;163:682 (Letter)

Fetal death, animal
Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss (Branch et al). 1990;163:210-6

Fetal development
Blood flow velocity and pulsatile diameter changes in the fetal descending aorta: a longitudinal study (Stale et al). 1990;163:26-9
Cocaine effects on fetal behavioral state (Tabor and Soffici) (Letter); (Hume) (Reply). 1990;163:1364-5
The differential neonatal morbidity of the intrauterine growth retardation syndrome (Villar et al). 1990;163:151-7
Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm (Hardardottir et al). 1990;163:1925-31
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66
Growth of linear parameters in trisomy 18 fetuses (Droste et al). 1990;163:158-61
Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns (Samaan et al). 1990;163:1836-9
Is indicated or spontaneous preterm delivery more advantageous for the fetus? (Owen et al). 1990;163:868-72
Metabolism of cocaine by human placentas: implications for fetal exposure (Roe et al). 1990;163:715-8
Neonatal morphometrics after endurance exercise during pregnancy (Clapp and Capeless). 1990;163:1805-11
Second-trimester ultrasonography: determinants of visualization of fetal anatomic structures (Catanzarite and Quirk). 1990;163:1191-5
Study of internal structure of the human fetus in utero by echo-planar magnetic resonance imaging (Johnson et al). 1990;163:601-7
Ultrasonographic fetal weight estimation by an integrated computer-assisted system: Can each laboratory improve its accuracy? (Thompson et al). 1990;163:986-95

Fetal development, animal
Dose response of thyrotropin-releasing hormone on pulmonary maturation in corticosteroid-treated preterm rabbits (Tabor et al). 1990;163:669-76
Effects of clonidine on breathing movements and electrocortical activity in the fetal lamb (Bamford et al). 1990;163:661-8
Embryopathic effects of short-term exposure to hypoglycemia in mouse embryos in vitro (Smoak and Sadler). 1990;163:619-24
Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb (Paulick et al). 1990;163:1357-63

Fetal diseases
Antenatal diagnosis of molybdenum cofactor deficiency (George et al). 1990;163:1203-4
Antenatal spontaneous perforation of the extrahepatic biliary tree (Chilukuri et al). 1990;163:1201-2
Diagnosis and management of prenatally detected myelomeningocele: a preliminary report (Hogge et al). 1990;163:1061-5
Prenatal diagnosis of fetal intracranial calcifications (Koga et al). 1990;163:1543-5
Second-trimester ultrasonography: determinants of visualization of fetal anatomic structures (Catanzarite and Quirk). 1990;163:1191-5
Spontaneous resolution of a nuchal fetal cystic hygroma diagnosed early in the second trimester of pregnancy (Meizner et al) (Letter); (Bronshstein and Blumenfeld) (Reply). 1990;163:267-9
Survival in a fetus with sacrococcygeal teratoma and hydrops (Nakayama). 1990;163:682 (Letter)
Ultrasonographic assessment of intestinal damage in fetuses with gastroschisis: Is it of clinical value? (Lenke et al). 1990;163:995-8

Fetal distress
Cerebral palsy and intrapartum fetal acidemia (Goodlin) (Letter); (Dennis et al) (Reply). 1990;163:266-7
Definition of fetal asphyxia (Clark) (Letter); (Johnson and Richards) (Reply). 1990;163:1364
Fetal heart rate monitoring during cesarean section (Dunn) (Letter); (Petrikovsky et al) (Reply). 1990;163:253

Fetal growth retardation
The association of maternal floor infarction of the placenta with adverse perinatal outcome (Andres et al). 1990;163:935-8
The differential neonatal morbidity of the intrauterine growth retardation syndrome (Villar et al). 1990;163:151-7
The effect of maternal hyperoxia on behavioral activity in growth-retarded human fetuses (Gagnon et al). 1990;163:1894-9
Effect of mitral valve prosthetic surgery on the outcome of a growth-retarded fetus: a case report (Izquierdo et al). 1990;163:584-7
The effect of race on the relationship between fetal death and altered fetal growth (Ferguson and Myers). 1990;163:1222-30
Growth of linear parameters in trisomy 18 fetuses (Droste et al). 1990;163:158-61
Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1990;163:1313-20
Is intrauterine transfusion associated with diminished fetal growth? (Utter et al). 1990;163:1781-4
Maternal serum α-fetoprotein levels in pregnancies complicated by diabetes: implications for screening programs (Martin et al). 1990;163:1209-16
Relationship of fetal biophysical profile and blood gas values at cordocentesis in severely growth-retarded fetuses (Ribbert et al). 1990;163:569-71

Fetal heart
Accuracy of apical four-chamber view as screen for congenital heart disease (Stephens). 1990;163:249-50 (Letter)
Acute fetal hemodynamic alterations after intrauterine

Fetal heart—cont'd
transfusion for treatment of severe red cell alloimmunization (Moise et al). 1990;163:776-84
Variation and correlation in human fetal umbilical Doppler velocities with fetal breathing: evidence of the cardiac-placental connection (Indik and Reed). 1990;163:1792-6
Fetal heart rate; *see* **Heart rate, fetal**
Fetal heart rate deceleration; *see* **Deceleration**
Fetal hypoxia; *see* **Fetal anoxia**
Fetal lung hypoplasia; *see* **Fetal organ maturity**
Fetal macrosomia; *see* **Macrosomia**
Fetal membranes
Cases with ruptured membranes that "reseal" (Johnson et al). 1990;163:1024-32
Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta (Cheung et al). 1990;163:1445-9
The role of fetal membranes in regulating production, transport, and metabolism of prostaglandin E_2 during labor (McCoshen et al). 1990;163:1632-40
Fetal membranes, premature rupture
Antibiotic therapy in preterm premature rupture of membranes: a randomized, prospective, double-blind trial (Johnston et al). 1990;163:743-7
Cases with ruptured membranes that "reseal" (Johnson et al). 1990;163:1024-32
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91
Gram stain results from amniocentesis in patients with preterm permature rupture of membranes—comparison of maternal and fetal characteristics (Asrat et al). 1990;163:887-9
Normal amniotic pressure in oligohydramnios after preterm rupture of membranes (Johnson and Maxwell) (Letter); (Nicolini et al) (Reply). 1990;163:1103-4
Perinatal survival with expectant management of midtrimester rupture of membranes (Major and Kitzmiller). 1990;163:838-44
Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study (Harger et al). 1990;163:130-7
Fetal membranes, prolonged rupture
Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes (Roberts and Mitchell). 1990;163:1560-6
Fetal monitoring
Acute fetal hemodynamic alterations after intrauterine transfusion for treatment of severe red cell alloimmunization (Moise et al). 1990;163:776-84
Cocaine effects on fetal behavioral state (Tabor and Soffici) (Letter); (Hume) (Reply). 1990;163:1364-5
The diagnostic values of concurrent nonstress testing, amniotic fluid measurement, and Doppler velocimetry in screening a general high-risk population (Devoe et al). 1990;163:1040-8
Effect of tocolytic agents on fetal umbilical velocimetry (Wright et al). 1990;163:748-50
Effects of hypertension on pregnancy monitoring and results (Lenox et al). 1990;163:1173-9
Fetal heart rate monitoring during cesarean section (Dunn) (Letter); (Petrikovsky et al) (Reply). 1990;163:253
The intrauterine sound environment of the human fetus during labor (Benzaquen et al). 1990;163:484-90
Relationship of fetal biophysical profile and blood gas values at cordocentesis in severely growth-retarded fetuses (Ribbert et al). 1990;163:569-71
Two-tier approach to biophysical assessment of the fetus (Mills et al). 1990;163:12-7
Fetal monitoring, animal
Effects of clonidine on breathing movements and electrocortical activity in the fetal lamb (Bamford et al). 1990;163:661-8
Fetal movement
Differential sensitivities of fetal muscle groups to *d*-tubocurarine (Szeto and Hinman). 1990;163:202-9
Effect of maternal-fetal movement on concentration of cells obtained at genetic amniocentesis (Carlan et al). 1990;163:490-3
Fetal eye movement assessed with real-time ultrasonography: Are there rapid and slow eye movements? (Horimoto et al). 1990;163:1480-4
Study results vary in count-to-10 method of fetal movement screening (Elbourne and Grant) (Letter); (Moore and Piacquadio) (Reply). 1990;163:264-5
Vibroacoustic stimulation and fetal behavioral state in normal term human pregnancy (Devoe et al). 1990;163:1156-61
Fetal organ maturity
Absence of evidence of pulmonary maturity at amniocentesis in term infants of diabetic mothers (Ojomo and Coustan). 1990;163:954-7
Blood flow velocity and pulsatile diameter changes in the fetal descending aorta: a longitudinal study (Stale et al). 1990;163:26-9
Digoxin-like immunoreactive factor and respiratory distress syndrome (Goodlin). 1990;163:1091 (Letter)
Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes (Roberts and Mitchell). 1990;163:1560-6
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66
Is indicated or spontaneous preterm delivery more advantageous for the fetus? (Owen et al). 1990;163:868-72
Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests (Kjos et al). 1990;163:898-903
Study of internal structure of the human fetus in utero by echo-planar magnetic resonance imaging (Johnson et al). 1990;163:601-7
Fetal organ maturity, animal
Dose response of thyrotropin-releasing hormone on pulmonary maturation in corticosteroid-treated preterm rabbits (Tabor et al). 1990;163:669-76
Effects of clonidine on breathing movements and electrocortical activity in the fetal lamb (Bamford et al). 1990;163:661-8
A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios (Harding et al). 1990;163:1904-13
Fetal outcome; *see* **Pregnancy outcome**
Fetal pulmonary hypoplasia; *see* **Fetal organ maturity**
Fetal sex determination; *see* **Sex determination**
Fetal umbilical velocimetry; *see* **Flowmeters**
Fetal viability
Does obstetric ethics have any role in the obstetrician's re-

sponse to the abortion controversy? (Chervenak and McCullough). 1990;163:1425-9 (Clin. opinion)
Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus (Carlson et al). 1990;163:1785-7

Fetofetal transfusion
Discrepancies in hemoglobin levels (Giles et al). 1990;163:1713 (Letter)
Twin-to-twin transfusion syndrome (Blickstein) (Letter); (Neilson) (Reply). 1990;163:677-8
Twin transfusion syndrome (Baldwin) (Letter); (Shah and Chaffin) (Reply). 1990;163:1376-7
Twin-twin transfusion syndrome (Urig et al). 1990;163:1522-6

Fetomaternal transfusion
The incidence of significant fetomaternal hemorrhage in patients undergoing cesarean section (Feldman et al). 1990;163:855-8

α-Fetoproteins; *see* **Alpha fetoproteins**

Fibrin
Enhanced generation and resolution of fibrin in women above the age of 30 years using oral contraceptives low in estrogen (Gram et al). 1990;163:438-42 (Supplement)

Fibrin fibrinogen degradation products
Enhanced generation and resolution of fibrin in women above the age of 30 years using oral contraceptives low in estrogen (Gram et al). 1990;163:438-42 (Supplement)

Fibrinogen
Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease (Kelleher). 1990;163:392-5 (Supplement)

Fibrinolysis
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Disorders of the hemostatic system and the risk of the development of thrombotic and cardiovascular diseases: limitations of laboratory diagnosis (Kluft). 1990;163:305-12 (Supplement)
Effects of long-term gestodene-containing oral contraceptive administration on hemostasis (Abbate et al). 1990;163:424-9 (Supplement)
Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid (Jespersen et al). 1990;163:396-403 (Supplement)
Hemostasis profile in women taking low-dose oral contraceptives (David et al). 1990;163:420-3 (Supplement)
Studies on the mechanism of action of oral contraceptives with regard to fibrinolytic variables (Kooistra et al). 1990;163:404-13 (Supplement)

Fibrinopeptide A
Effects of long-term gestodene-containing oral contraceptive administration on hemostasis (Abbate et al). 1990;163:424-9 (Supplement)

Fibroids, uterine; *see* **Uterine neoplasms**

Fingerprinting, DNA; *see* **Nucleotide mapping**

Fingerprints, nucleotide; *see* **Nucleotide mapping**

Fistula, vesicovaginal; *see* **Vesicovaginal fistula**

Flow cytometry
Flow cytometric quantitation of tumor-associated antigens in solid tumors (van Dam et al) (Letter); (Nozawa) (Reply). 1990;163:698-9
Lectins and heparin-binding features of human spermatozoa as analyzed by flow cytometry (Vasquez et al). 1990;163:2006-12

Flowmeters
Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry (Ikeda and Mori). 1990;163:494-8
Color flow Doppler—a useful instrument in the diagnosis of vasa previa (Harding et al). 1990;163:1566-8
The diagnostic values of concurrent nonstress testing, amniotic fluid measurement, and Doppler velocimetry in screening a general high-risk population (Devoe et al). 1990;163:1040-8
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66
The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term (Moretti et al). 1990;163:1844-8
Effect of tocolytic agents on fetal umbilical velocimetry (Wright et al). 1990;163:748-50
Fetal Doppler velocimetry in twins (Degani) (Letter); (Divon) (Reply). 1990;163:1100-1
Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive value for pregnancy-induced hypertension (Hume et al). 1990;163:826-9
Prenatal diagnosis of a chest wall mass with ultrasonography and Doppler velocimetry: a case report (Smith et al). 1990;163:567-9
Variation and correlation in human fetal umbilical Doppler velocities with fetal breathing: evidence of the cardiac-placental connection (Indik and Reed). 1990;163:1792-6

Fluorouracil
5-Fluorouracil exposure during the period of conception: report on two cases (Odom et al). 1990;163:76-7

Focal migraine; *see* **Migraine**

Follicle, Graafian; *see* **Graafian follicle**

Follicle stimulating hormone; *see* **FSH**

Forceps, obstetrical; *see* **Obstetrical forceps**

Foreign bodies
A vesicovaginal fistula and intravesical foreign body: a rare case of the neglected pessary (Goldstein et al). 1990;163:589-91

FSH
The luteinizing hormone–releasing hormone pulse generator in men: abnormalities and clinical management (Matsumoto et al). 1990;163:1743-51 (Supplement)
Relationship between plasma calcium fractions, other bone-related variables, and serum follicle-stimulating hormone levels in premenopausal, perimenopausal, and postmenopausal women (Nordin et al). 1990;163:140-5

5-FU; *see* **Fluorouracil**

G

Gastrointestinal diseases
Gastrointestinal disease and oral contraception (Hanker). 1990;163:2204-7 (Symposium)

Gastroschisis; *see* **Abdominal wall**

Gastrula
Embryopathic effects of short-term exposure to hypoglycemia in mouse embryos in vitro (Smoak and Sadler). 1990;163:619-24

Genetic screening
Appropriate screening tests (Nagey) (Letter); (Chervenak et al) (Reply). 1990;163:688-9
Genetics
Sharing of human leukocyte antigens in primary and secondary recurrent spontaneous abortions (Ho et al). 1990;163:178-88
Genital neoplasms, female
The Maylard incision in gynecologic cancer (Helmkamp and Krebs). 1990;163:1554-7
Genome, human
Isolation and characterization of the gene from a human genome encoding 17β-estradiol dehydrogenase: a comparison of Jar and BeWo choriocarcinoma cell lines (Long et al). 1990;163:1976-81
Gestagens; *see* **Sex hormones**
Gestational age
Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants (Mills et al). 1990;163:1568-74
Ultrasonographic dating of very early pregnancy (Cadkin and McAlpin-Cadkin). 1990;163:1103 (Letter)
Ultrasonographic fetal weight estimation by an integrated computer-assisted system: Can each laboratory improve its accuracy? (Thompson et al). 1990;163:986-95
Gestational diabetes; *see* **Pregnancy in diabetes**
Gestodene
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Clinical acceptability of monophasic gestodene (Düsterberg and Brill). 1990;163:1398-403 (Supplement)
Inhibition of oral contraceptive steroid-metabolizing enzymes by steroids and drugs (Guengerich). 1990;163:2159-63 (Symposium)
Inhibition of ovulation by low-dose monophasic contraceptive containing gestodene (Thomas and Vankrieken). 1990;163:1404-9 (Supplement)
Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism (Jung-Hoffmann and Kuhl). 1990;163:2183-97 (Symposium)
Pharmacokinetics of gestodene and ethinyl estradiol after oral administration of a monophasic contraceptive (Täuber et al). 1990;163:1414-20 (Supplement)
Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women (Kuhnz). 1990;163:2120-7 (Symposium)
Gestodene/ethinyl estradiol
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Effects of long-term gestodene-containing oral contraceptive administration on hemostasis (Abbate et al). 1990;163:424-9 (Supplement)
Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid (Jespersen et al). 1990;163:396-403 (Supplement)
Estrogenic effect of gestodene- or desogestrel-containing oral contraceptives on lipoprotein metabolism (Gevers Leuven et al). 1990;163:358-62 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives (Kuhl et al). 1990;163:363-9 (Supplement)
Gittes procedure; *see* **Suspensions**
Glucose
Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor (Romero et al). 1990;163:968-74
Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level (Skouby et al). 1990;163:343-8 (Supplement)
Response of placental vasculature to high glucose levels in the isolated human placental cotyledon (Roth et al). 1990;163:1828-30
Glucose tolerance test
Does fasting interval affect the glucose challenge test? (Berkus et al). 1990;163:1812-7
Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: results of a controlled, randomized, prospective study (Kjos et al). 1990;163:1822-7
Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial (Jandrain et al). 1990;163:378-81 (Supplement)
Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months post partum (Kjos et al). 1990;163:93-8
Glucose tolerance in pregnancy: ethnic variation and influence of body habitus (Green et al). 1990;163:86-92
Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system (Beck-Nielsen et al). 1990;163:292-5 (Supplement)
Low-dose oral contraceptives and carbohydrate metabolism (Godsland et al). 1990;163:348-53 (Supplement)
Relationships between glucose levels and insulin secretion during a glucose challenge test (Berkus et al). 1990;163:1818-21
Glycoprotein hormones, alpha subunit
Stability of human chorionic gonadotropin and its α-subunit (Rao et al). 1990;163:1098-9 (Letter)
Glycoproteins
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84
Expression of P-glycoprotein in epithelial ovarian cancer: evaluation as a marker of multidrug resistance (Rubin et al). 1990;163:69-73
Glycosylation
Comparisons of glycosylated and nonglycosylated amniotic fluid prolactins in normal second- and third-trimester pregnancies (McCoshen et al). 1990;163:81-5
GnRH; *see* **Pituitary hormone releasing hormones**
Gonadal dysgenesis
Correlation of the testicular determinant factor sequence zinc finger Y with varying gonadal phenotypes in a series

of 13 subjects with gonadal dysgenesis because of Y aneuploidy (Tho et al). 1990;163:1968-75

Gonadotropin releasing hormone agonists; *see* **Pituitary hormone release inhibiting hormones**

Gonadotropin releasing hormones; *see* **Pituitary hormone releasing hormones**

Gonadotropins, chorionic

Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels (Kratzer and Taylor). 1990;163:1497-502

Maternal serum Down syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin (Macri et al). 1990;163:1248-53

Measures of test performance and predictive values (Kadar) (Letter); (Schiff et al) (Reply). 1990;163:1374-5

Use of serial human chorionic gonadotropin measurements to differentiate between intrauterine and ectopic pregnancies (Kadar) (Letter); (Lindblom et al) (Reply). 1990;163:259-61

Graafian follicle

Oxytocin and vasopressin binding sites in human and bovine ovaries (Fuchs et al). 1990;163:1961-7

Gram stain; *see* **Stains and staining**

Granulosa cell tumor

Large cystic granulosa cell tumor: case report (Choi and Pritchard). 1990;163:74-6

Granulosa cell tumor, animal

Ovarian granulosa cell tumorigenesis in SWR-derived F1 hybrid mice: preneoplastic follicular abnormality and malignant disease progression (Tennent et al). 1990;163:625-34

Group B streptococcus; *see* ***Streptococcus agalactiae***

Growth factors; *see* **Growth substances**

Growth retardation, intrauterine; *see* **Fetal growth retardation**

Growth substances

Growth factor activity in the blood of women in whom preeclampsia develops is elevated from early pregnancy (Taylor et al). 1990;163:1839-44

Preferential mitogenic activity for myoblast-like cells can be extracted from uterine leiomyoma tissues (Koutsilieris et al). 1990;163:1665-70

Gynecologic procedures; *see* **Surgery, operative**

H

Habitual abortion; *see* **Abortion, habitual**

HBsAg; *see* **Hepatitis B surface antigens**

Heart defects, congenital

Accuracy of apical four-chamber view as screen for congenital heart disease (Stephens). 1990;163:249-50 (Letter)

Heart failure, congestive

In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1

Heart rate, fetal

Effects of hypertension on pregnancy monitoring and results (Lenox et al). 1990;163:1173-9

Effects of intravascular fetal blood transfusion on fetal intracardiac Doppler velocity waveforms (Rizzo et al). 1990;163:1231-8

Fetal heart rate monitoring during cesarean section (Dunn) (Letter); (Petrikovsky et al) (Reply). 1990;163:253

Graphic mnemonic for variable decelerations (Chao). 1990;163:1098 (Letter)

Sinusoidal fetal heart rate pattern during cardiopulmonary bypass (Burke et al). 1990;163:17-8

Sources of variability in umbilical artery systolic/diastolic ratios: implications of Poiseuille equation (Wright and Ridgway). 1990;163:1788-91

Vibroacoustic stimulation and fetal behavioral state in normal term human pregnancy (Devoe et al). 1990;163:1156-61

Heart rate, fetal, animal

Role of vasopressin in mediation of fetal cardiovascular responses to acute hypoxia (Piacquadio et al). 1990;163:1294-300

Heart septum

Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy (van Eyck et al). 1990;163:1239-42

Heart transplantation

Postpartum coronary artery dissection followed by heart transplantation (Curiel et al). 1990;163:538-9

Heart valve prosthesis

Effect of mitral valve prosthetic surgery on the outcome of a growth-retarded fetus: a case report (Izquierdo et al). 1990;163:584-7

Heart ventricle, animal

Nifedipine enhances the cardiac toxicity of magnesium sulfate in the isolated perfused Sprague-Dawley rat heart (Thorp et al). 1990;163:655-6

HELLP syndrome; *see* **Pregnancy complications, hematologic**

Hemangioma

Prenatal diagnosis of a chest wall mass with ultrasonography and Doppler velocimetry: a case report (Smith et al). 1990;163:567-9

Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis (Chazotte et al). 1990;163:1180-1

Hemodynamics

Acute fetal hemodynamic alterations after intrauterine transfusion for treatment of severe red cell alloimmunization (Moise et al). 1990;163:776-84

Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry (Ikeda and Mori). 1990;163:494-8

Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat (Mabie et al). 1990;163:1861-7

Hemoglobin A, glycosylated

Maternal serum α-fetoprotein levels in pregnancies complicated by diabetes: implications for screening programs (Martin et al). 1990;163:1209-16

Hemoglobins

Discrepancies in hemoglobin levels (Giles et al). 1990;163:1713 (Letter)

Hemolytic disease of newborn; *see* **Erythroblastosis, fetal**

Hemoperitoneum

Hemoperitoneum as a result of coital injury without associated vaginal injury (McColgin et al). 1990;163:1503-5

Hemorrhage

A comment on thumbtacks for hemorrhage control (Mann) (Letter); (Patsner) (Reply). 1990;163:1092-3

Hyperadrenergic orthostatic intolerance as a result of postpartum blood loss (Mader et al). 1990;163:1578-9

A reappraisal of the need for autologous blood donation in the obstetric patient (Andres et al). 1990;163:1551-3

Hemostasis
Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease (Kelleher). 1990;163:392-5 (Supplement)
A comment on thumbtacks for hemorrhage control (Mann) (Letter); (Patsner) (Reply). 1990;163:1092-3
Hemostasis profile in women taking low-dose oral contraceptives (David et al). 1990;163:420-3 (Supplement)
Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease (Juhan-Vague and Vague). 1990;163:313-5 (Supplement)
Oral contraceptives in risk groups: Exclusion or monitoring? (Guillebaud). 1990;163:443-6 (Supplement)
Hemostatic system disorders; *see* **Blood coagulation disorders**
Heparin
Lectins and heparin-binding features of human spermatozoa as analyzed by flow cytometry (Vasquez et al). 1990;163:2006-12
Hepatitis B
Intrapartum hepatitis B screening in a low-risk population (Ernest et al). 1990;163:978-80
Hepatitis B surface antigens
HBcAg identification in the placental cytotypes of symptom-free HBsAg-carrier mothers: a study with the immunoperoxidase method (Lucifora et al). 1990;163:235-9
Intrapartum hepatitis B screening in a low-risk population (Ernest et al). 1990;163:978-80
Hepatocytes; *see* **Liver**
Hepatolenticular degeneration
Pregnancy in a patient with treated Wilson's disease: a case report (Dupont et al). 1990;163:1527-8
Hernia, umbilical
The associated anomalies that determine prognosis in congenital omphaloceles (Tucci and Bard). 1990;163:1646-9
Fetal gastroschisis and omphalocele: Is cesarean section the best mode of delivery? (Lewis et al). 1990;163:773-5
Heterotopic pregnancy; *see* **Pregnancy, ectopic**
Hippel-Lindau disease
Papillary cystadenoma of the broad ligament in von Hippel-Lindau disease (Korn et al). 1990;163:596-8
Hirsutism
Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women (Wild et al). 1990;163:1998-2005
Histamine
Studies of the spontaneous motility and the effect of histamine on isolated myometrial strips of the nonpregnant human uterus: the influence of various uterine abnormalities (Martinez-Mir et al). 1990;163:189-95
HIV
Pregnancy outcomes among mothers infected with human immunodeficiency virus and uninfected control subjects (Minkoff et al). 1990;163:1598-604
The relationship of cocaine use to syphilis and human immunodeficiency virus infections among inner city parturient women (Minkoff et al). 1990;163:521-6
Risk factors associated with the increasing prevalence of pneumonia during pregnancy (Berkowitz and LaSala). 1990;163:981-5
Vulvar ulcer of unknown etiology in a human immunodeficiency virus–infected woman: response to treatment with zidovudine (Covino and McCormack). 1990;163:116-8
HIV infections
Drug prophylaxis for human immunodeficiency virus–infected pregnant women: ethical considerations (Minkoff and Moreno). 1990;163:1111-4 (Clin. opinion)
Sexually transmitted diseases and human immunodeficiency virus infection among women with pelvic inflammatory disease (Hoegsberg et al). 1990;163:1135-9
HLA antigens
Sharing of human leukocyte antigens in primary and secondary recurrent spontaneous abortions (Ho et al). 1990;163:178-88
HLA-DR1 antigen
Interleukin-1β, human leukocyte antigen DR1, and transforming growth factor-β expression in endometrium, placenta, and placental membranes (Kauma et al). 1990;163:1430-7
Hormone–binding globulin
Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin (Bergink et al). 1990;163:2132-7 (Symposium)
Human chorionic gonadotropin; *see* **Gonadotropins, chorionic**
Human chorionic gonadotropin, alpha subunit; *see* **Glycoprotein hormones, alpha subunit**
Human immunodeficiency virus; *see* **HIV**
Human leukocyte antigen; *see* **HLA antigens**
Human leukocyte antigen DR1; *see* **HLA-DR1 antigen**
Hybridization
Preembryo biopsy and analysis of blastomeres by in situ hybridization (Grifo et al). 1990;163:2013-9
The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes (Lewandowski et al). 1990;163:1333-7
Hydatidiform mole
Differential diagnosis between complete mole and hydropic abortus by deoxyribonucleic acid fingerprints (Nobunaga et al). 1990;163:634-8
Discrepancy in the diagnoses of hydatidiform mole by macroscopic and microscopic findings and the deoxyribonucleic acid fingerprint method (Takahashi et al). 1990;163:112-3
Hydramnios
Indomethacin treatment for polyhydramnios and subsequent infantile nephrogenic diabetes insipidus (Smith et al). 1990;163:98-9
Observations on the cause of oligohydramnios in prolonged pregnancy (Trimmer et al). 1990;163:1900-3
The stuck twin phenomenon: Ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses (Mahony et al). 1990;163:1513-22
Superiority of the four-quadrant sum over the single-deepest-pocket technique in ultrasonographic identification of abnormal amniotic fluid volumes (Moore). 1990;163:762-7
Twin-twin transfusion syndrome (Urig et al). 1990;163:1522-6
Hydramnios, animal
A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios (Harding et al). 1990;163:1904-13

Hydrocortisone
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Hydrogen-ion concentration
Comparison of umbilical artery pH and 5-minute Apgar score in the low-birth-weight and very-low-birth-weight infant (Stark et al). 1990;163:818-23
Hydropic abortus; *see* **Hydrops fetalis**
Hydrops, nonimmune; *see* **Edema**
Hydrops fetalis
Differential diagnosis between complete mole and hydropic abortus by deoxyribonucleic acid fingerprints (Nobunaga et al). 1990;163:634-8
Management and outcomes of pregnancies complicated by human B19 parvovirus infection: a prospective study (Rodis et al). 1990;163:1168-71
Niemann-Pick disease associated with nonimmune hydrops fetalis (Meizner et al). 1990;163:128-9
Placental transfer of cefazolin and piperacillin in pregnancies remote from term complicated by Rh isoimmunization (Brown et al). 1990;163:938-43
Prenatal diagnosis of laryngeal atresia (Watson et al). 1990;163:1456-7
Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus (Carlson et al). 1990;163:1785-7
Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis (Chazotte et al). 1990;163:1180-1
Survival in a fetus with sacrococcygeal teratoma and hydrops (Nakayama). 1990;163:682 (Letter)
Hydroxyprostaglandin dehydrogenases
Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta (Cheung et al). 1990;163:1445-9
Hygroma, cystic; *see* **Lymphangioma**
Hygroscopic cervical dilators; *see* **Surgical instruments**
Hyperandrogenism; *see* **Hirsutism**
Hyperbaric oxygenation
Hyperbaric oxygen therapy for air embolism complicating operative hysteroscopy (Tur-Kaspa) (Letter); (Baggish) (Reply). 1990;163:680-1
Hyperimmunoglobulinemia; *see* **Immunoglobulins**
Hyperkalemia
Hyperkalemia secondary to irradiation of packed red blood cells: possible effects with intravascular fetal transfusion (Thorp et al). 1990;163:607-9
Hypertension
Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986 (Saftlas et al). 1990;163:460-5
Fetal and maternal response to intravenous infusion of a thromboxane synthetase inhibitor (Cardin et al). 1990;163:1345-9
Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive value for pregnancy-induced hypertension (Hume et al). 1990;163:826-9
Intracellular sodium levels in pregnant women with hypertension (Goodlin) (Letter); (Sowers and Zemel) (Reply). 1990;163:261-2
Laboratory identification of the lupus anticoagulant in normal pregnancy and pregnancy-induced hypertension (Riveros et al) (Letter); (Lockwood) (Reply). 1990;163:704-5
Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia (August et al). 1990;163:1612-21
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. 1990;163:1691-712 (Supplement)
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy: foreword (Lenfant et al). 1990;163:1689-90 (Supplement)
The natural history of thrombocytopenia associated with preeclampsia (Katz et al). 1990;163:1142-3
Oral contraception in disease states (Breckwoldt et al). 1990;163:2213-6 (Symposium)
Hypertension, animal
Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1990;163:1313-20
Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat (Mabie et al). 1990;163:1861-7
Hyperviscosity, animal; *see* **Blood viscosity, animal**
Hypoglycemia, animal
Embryopathic effects of short-term exposure to hypoglycemia in mouse embryos in vitro (Smoak and Sadler). 1990;163:619-24
Hypogonadism
Gonadotropin-releasing hormone deficiency in men: diagnosis and treatment with exogenous gonadotropin-releasing hormone (Crowley and Whitcomb). 1990;163:1752-8 (Supplement)
Hypotension
Effect of uterine contractility and maternal hypotension on prolonged decelerations after bupivacaine epidural anesthesia (Steiger and Nageotte). 1990;163:808-12
Hypotension, animal
Magnesium sulfate and promethazine do not interact to cause hypotension in gravid ewes (Pollack et al). 1990;163:787-94
Hypotension, orthostatic
Hyperadrenergic orthostatic intolerance as a result of postpartum blood loss (Mader et al). 1990;163:1578-9
Hypothalamus
Parameters of hypothalamic aging (Seifer and Jones) (Letter); (Alexander and Aksel) (Reply). 1990;163:1715
Hypothyroidism
Early hypothyroidism in patients with menorrhagia (Blesing and Hambley) (Letter); (Wilansky and Greisman) (Reply). 1990;163:697
Screening infertile women for subclinical hypothyroidism (Strickland et al) (Letter); (Wilansky and Greisman) (Reply). 1990;163:262-3
Hypoxia; *see* **Anoxia**
Hypoxia, fetal; *see* **Fetal anoxia**
Hyskon, adverse effects
Explanation of mechanism of Hyskon solution reaction needed (Loffer). 1990;163:2029 (Letter)
Hysterectomy
Bacterial vaginosis and trichomoniasis vaginitis are risk fac-

Hysterectomy—cont'd
tors for cuff cellulitis after abdominal hysterectomy (Soper et al). 1990;163:1016-23
Experience with the EndoPap device for the cytologic detection of uterine cancer and its precursors: a comparison of the EndoPap with fractional curettage or hysterectomy (LaPolla et al). 1990;163:1055-60
The impact of a quality assurance process on the frequency and confirmation rate of hysterectomy (Gambone et al). 1990;163:545-50
Hysteroscopy
Hyperbaric oxygen therapy for air embolism complicating operative hysteroscopy (Tur-Kaspa) (Letter); (Baggish) (Reply). 1990;163:680-1
Safety of 32% dextran 70 for hysteroscopy (Ljungström) (Letter); (Jedeikin and Olsfanger) (Reply). 1990;163:2029-30
Hysteroscopy, animal
A rabbit model for bacteria-induced preterm pregnancy loss (Dombroski et al). 1990;163:1938-43

I

Idiopathic hypogonadotropic hypogonadism; *see* **Hypogonadism**
Idiopathic thrombocytopenia purpura; *see* **Purpura, thrombocytopenic**
IgG
Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss (Branch et al). 1990;163:210-6
Immune globulin; *see* **Immunization, passive**
Immune sera
A case of Rh isoimmunization: Should threatened first-trimester abortion be an indication for Rh immune globulin prophylaxis? (Dayton et al). 1990;163:63-4
Immunity, cellular
Lymphocyte proliferative response to fertilization antigen in patients with antisperm antibodies (Naz et al). 1990;163:610-3
Immunization, passive
A case of Rh isoimmunization: Should threatened first-trimester abortion be an indication for Rh immune globulin prophylaxis? (Dayton et al). 1990;163:63-4
Postinjection kinetics of antepartum Rh immune globulin (Witter et al). 1990;163:784-6
Immunoassay
A comparison of sperm antibody assays (Daru and Mathur). 1990;163:1622-9
Immunocontraception; *see* **Contraception, immunologic**
Immunocyte chemistry; *see* **Immunohistochemistry**
Immunoenzyme techniques
Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients (Towers et al). 1990;163:965-7
HBcAg identification in the placental cytotypes of symptom-free HBsAg-carrier mothers: a study with the immunoperoxidase method (Lucifora et al). 1990;163:235-9
Immunoglobulin G; *see* **IgG**
Immunoglobulins
Continuing controversy in alloimmune thrombocytopenia: fetal hyperimmunoglobulinemia fails to prevent thrombocytopenia (Nicolini et al). 1990;163:1144-6
Immunohistochemistry
Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta (Cheung et al). 1990;163:1445-9
Uterine artery estrogen receptors in the nonpregnant and pregnant guinea pig (Leiberman et al). 1990;163:1685-8
Immunoperoxidase methods; *see* **Immunoenzyme techniques**
Immunosuppression
Soluble factors produced by isolated first-trimester chorionic villi directly inhibit proliferation of T cells (Silver et al). 1990;163:1914-9
Impedance cardiography; *see* **Cardiography, impedance**
In situ hybridization; *see* **Hybridization**
In vitro fertilization; *see* **Fertilization in vitro**
Incisions; *see* **Surgery, operative**
Incontinence, urinary, stress; *see* **Urinary incontinence, stress**
Indomethacin
Indomethacin treatment for polyhydramnios and subsequent infantile nephrogenic diabetes insipidus (Smith et al). 1990;163:98-9
Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb (Paulick et al). 1990;163:1357-63
Induced labor; *see* **Labor, induced**
Infant, low birth weight
Comparison of umbilical artery pH and 5-minute Apgar score in the low-birth-weight and very-low-birth-weight infant (Stark et al). 1990;163:818-23
The effect of physical activity during pregnancy on preterm delivery and birth weight (Klebanoff et al). 1990;163:1450-6
Mortality rates ignore population segment (Harkavy) (Letter); (Resnick and Ariet) (Reply). 1990;163:269-70
The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening (Gillogley et al). 1990;163:1535-42
Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33
Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants (Mills et al). 1990;163:1568-74
Survival and outcome of infants weighing <800 grams at birth (Lipper et al). 1990;163:146-50
Infant, newborn
The association of intrapartum asphyxia in the mature fetus with newborn behavior (Low et al). 1990;163:1131-5
Correlations between antepartum maternal metabolism and newborn behavior (Rizzo et al). 1990;163:1458-64
Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns (Samaan et al). 1990;163:1836-9
Infant, newborn, diseases
Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients (Towers et al). 1990;163:965-7
Cost-effectiveness of intrapartum screening and treatment for maternal group B streptococci colonization (Strickland et al). 1990;163:4-8 (Clin. opinion)
The differential neonatal morbidity of the intrauterine growth retardation syndrome (Villar et al). 1990;163:151-7
Digoxin-like immunoreactive factor and respiratory distress syndrome (Goodlin). 1990;163:1091 (Letter)

In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1
Indomethacin treatment for polyhydramnios and subsequent infantile nephrogenic diabetes insipidus (Smith et al). 1990;163:98-9

Infant, premature
Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor (Romero et al). 1990;163:968-74
Antenatal microbiologic and maternal risk factors associated with prematurity (McGregor et al). 1990;163:1465-73
Calcium supplementation during pregnancy may reduce preterm delivery in high-risk populations (Villar and Repke). 1990;163:1124-31
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91
Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population (Owen et al). 1990;163:873-9
Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants (Mills et al). 1990;163:1568-74
Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study (Harger et al). 1990;163:130-7

Infant behavior; *see* **Behavior, infant**

Infant mortality
A chill wind blows: Webster, obstetrics, and the health of women (Chavkin and Rosenfield). 1990;163:450-2 (Clin. opinion)
Mortality rates ignore population segment (Harkavy) (Letter); (Resnick and Ariet) (Reply). 1990;163:269-70
Perinatal mortality in Victoria, Australia: role of group B streptococcus (Fliegner and Garland). 1990;163:1609-11
Preeclampsia in diabetic pregnancies (Garner et al). 1990;163:505-8
Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33
Survival rates of monoamniotic twins do not decrease after 30 weeks' gestation (Carr et al). 1990;163:719-22

Infarction
The association of maternal floor infarction of the placenta with adverse perinatal outcome (Andres et al). 1990;163:935-8

Infection
Gram stain results from amniocentesis in patients with preterm permature rupture of membranes—comparison of maternal and fetal characteristics (Asrat et al). 1990;163:887-9
HBcAg identification in the placental cytotypes of symptom-free HBsAg-carrier mothers: a study with the immunoperoxidase method (Lucifora et al). 1990;163:235-9
Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in twin gestations with preterm labor (Romero et al). 1990;163:757-61
Preterm delivery: a risk factor for retained placenta (Romero et al). 1990;163:823-5

Infection, animal
A rabbit model for bacteria-induced preterm pregnancy loss (Dombroski et al). 1990;163:1938-43

Infection, congenital
Prenatal diagnosis of congenital cytomegalovirus infection: two decades later (Grose and Weiner). 1990;163:447-50 (Clin. opinion)

Infertility, female
Antisperm antibodies in women undergoing intrauterine insemination (Goldberg et al). 1990;163:65-8
Association between autoantibodies and unexplained infertility unproved (Maier) (Letter); (Taylor and Campbell) (Reply). 1990;163:243
Human oocyte and preembryo donation: an evolving method for the treatment of infertility (Sauer and Paulson). 1990;163:1421-4 (Clin. opinion)
Infertility and eating disorders (Stewart et al). 1990;163:1196-9
Screening infertile women for subclinical hypothyroidism (Strickland et al) (Letter); (Wilansky and Greisman) (Reply). 1990;163:262-3
The use of serial CA 125 concentrations to monitor endometriosis in infertile women (Pittaway). 1990;163:1032-7

Infertility, male
A comparison of sperm antibody assays (Daru and Mathur). 1990;163:1622-9

Inflammatory bowel disease
Intrapartum uterine rupture and inflammatory bowel disease (Peterson and Braak). 1990;163:1247-8

Informed consent
Drug prophylaxis for human immunodeficiency virus–infected pregnant women: ethical considerations (Minkoff and Moreno). 1990;163:1111-4 (Clin. opinion)

Insemination, artificial
Antisperm antibodies in women undergoing intrauterine insemination (Goldberg et al). 1990;163:65-8

Instruments, surgical; *see* **Surgical instruments**

Insulin
Does fasting interval affect the glucose challenge test? (Berkus et al). 1990;163:1812-7
Effects of ethinyl estradiol combined with desogestrel and cyproterone acetate on glucose tolerance and insulin response to an oral glucose load: a one-year randomized, prospective, comparative trial (Jandrain et al). 1990;163:378-81 (Supplement)
Infants of diabetic mothers with accelerated fetal growth by ultrasonography: Are they all alike? (Keller et al). 1990;163:893-7
Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease (Juhan-Vague and Vague). 1990;163:313-5 (Supplement)
Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors (Devaskar et al). 1990;163:1350-6
Low-dose oral contraceptives and carbohydrate metabolism (Godsland et al). 1990;163:348-53 (Supplement)
Relationships between glucose levels and insulin secretion during a glucose challenge test (Berkus et al). 1990;163:1818-21
Suppression of serum insulin level by diazoxide does not alter serum testosterone of sex hormone–binding globulin levels in healthy, nonobese women (Nestler et al). 1990;163:1243-6

Insulin-dependent diabetes; *see* **Pregnancy in diabetes**

Insulin-like growth factor I
Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis (Nagamani and Stuart). 1990;163:1992-7

Insulin-like growth factor II
Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns (Samaan et al). 1990;163:1836-9
Insulin resistance
Diabetic ketoacidosis and insulin resistance with subcutaneous terbutaline infusion: a case report (Tibaldi et al). 1990;163:509-10
Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom Study (Simon et al). 1990;163:382-7 (Supplement)
Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system (Beck-Nielsen et al). 1990;163:292-5 (Supplement)
Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level (Skouby et al). 1990;163:343-8 (Supplement)
Intensive care, neonatal
Mortality rates ignore population segment (Harkavy) (Letter); (Resnick and Ariet) (Reply). 1990;163:269-70
Survival and outcome of infants weighing <800 grams at birth (Lipper et al). 1990;163:146-50
Interferon-γ; *see* **Interferon type II**
Interferon type II
Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies (Mutch et al). 1990;163:1920-4
Interleukin-1
Mechanism of action of interleukin-1β in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells (Petraglia et al). 1990;163:1307-12
Interleukin-2
Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells (Dudley et al). 1990;163:1890-3
Serum levels of interleukin-2 and tumor necrosis factor α in women with recurrent abortion (Mallmann et al). 1990;163:1367 (Letter)
Interleukins
Interleukin-1β, human leukocyte antigen DR1, and transforming growth factor-β expression in endometrium, placenta, and placental membranes (Kauma et al). 1990;163:1430-7
Internal carotid artery; *see* **Carotid artery, internal**
Internship and residency
Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? (Chervenak and McCullough). 1990;163:1425-9 (Clin. opinion)
Intraamniotic infection; *see* **Amniotic fluid**; **Chorioamnionitis**
Intracellular fluid
Intracellular sodium levels in pregnant women with hypertension (Goodlin) (Letter); (Sowers and Zemel) (Reply). 1990;163:261-2
Intrapartum asphyxia; *see* **Fetal anoxia**
Intrauterine devices
Cardiovascular death among women under 40 years of age using low-estrogen oral contraceptives and intrauterine devices in Finland from 1975 to 1984 (Hirvonen and Idänpään-Heikkilä). 1990;163:281-4 (Supplement)
Contraception and ectopic pregnancy risk (Franks et al). 1990;163:1120-3
Laparoscopic ventrosuspensions (Yoong). 1990;163:1151-3
Ovarian pregnancy: a report of 20 cases in one institution (Raziel et al). 1990;163:1182-5
Intrauterine growth retardation. Fetal growth retardation; *see* **Fetal growth retardation**
Intrauterine synechiae; *see* **Adhesions**
Intrauterine transfusion; *see* **Blood transfusion, intrauterine**
Ionized calcium; *see* **Calcium**
Irrigation
Smoking and the antioxidant ascorbic acid: plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women (Basu et al). 1990;163:1948-52
Islands of Langerhans
Anencephalics as organ donors (Van Assche). 1990;163:599-600
Isoenzymes
Effects of ritodrine tocolysis on cardiac isozymes and electrocardiography (Hendricks and Katz) (Letter); (Hadi and Albazzaz) (Reply). 1990;163:699-701
Isradipine
Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy (Skajaa et al). 1990;163:1323-33
Items
Items. 1990;163:272, 714, 1108, 1378, 1718, 2032

J

Jurisprudence
A chill wind blows: Webster, obstetrics, and the health of women (Chavkin and Rosenfield). 1990;163:450-2 (Clin. opinion)

K

Karyotype abnormalities; *see* **Chromosome abnormalities**
Karyotyping
Effect of maternal-fetal movement on concentration of cells obtained at genetic amniocentesis (Carlan et al). 1990;163:490-3
Second-trimester placental biopsy for rapid fetal karyotyping (Cameron et al). 1990;163:931-4
Ketoconazole
Ketoconazole treatment of Cushing's syndrome in pregnancy (Divers). 1990;163:1101 (Letter)
Ketosis, diabetic
Diabetic ketoacidosis and insulin resistance with subcutaneous terbutaline infusion: a case report (Tibaldi et al). 1990;163:509-10
Kidney failure, chronic
Chronic renal disease and pregnancy outcome (Cunningham et al). 1990;163:453-9

L

Labor
Changes in active and latent collagenase in human placenta around the time of parturition (Rajabi et al). 1990;163:499-503
A comparison of the effect of epidural, general, and no anesthesia on funic acid-base values by stage of labor and type of delivery (Shyken et al). 1990;163:802-7
The course of labor after endurance exercise during pregnancy (Clapp). 1990;163:1799-805
The intrauterine sound environment of the human fetus during labor (Benzaquen et al). 1990;163:484-90
Labor epidural analgesia and dystocia-related cesarean section (Downing and Knape). 1990;163:1096-7 (Letter)

The role of fetal membranes in regulating production, transport, and metabolism of prostaglandin E_2 during labor (McCoshen et al). 1990;163:1632-40

Labor, induced

Double-blind comparison of intravaginal prostaglandin E_2 gel and "chip" for preinduction cervical ripening (Smith et al). 1990;163:845-7

Induction of labor with mifepristone (RU 486) in intrauterine fetal death (Cabrol et al). 1990;163:540-2

Induction of labor with pulsatile oxytocin (Cummiskey and Dawood). 1990;163:1868-74

Labor, induced, animal

Effect of intravaginal application of prostaglandin E_2 gel on the mechanical properties of the ovine cervix uteri at term (Owiny and Fitzpatrick). 1990;163:657-60

Labor, premature

Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor (Romero et al). 1990;163:968-74

Antenatal microbiologic and maternal risk factors associated with prematurity (McGregor et al). 1990;163:1465-73

Awaiting cervical change for the diagnosis of preterm labor does not compromise the efficacy of ritodrine tocolysis (Utter et al). 1990;163:882-6

A comparison of tocolysis with nifedipine or ritodrine: analysis of efficacy and maternal, fetal, and neonatal outcome (Ferguson et al). 1990;163:105-11

The effect of physical activity during pregnancy on preterm delivery and birth weight (Klebanoff et al). 1990;163:1450-6

Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population (Owen et al). 1990;163:873-9

Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in twin gestations with preterm labor (Romero et al). 1990;163:757-61

Is indicated or spontaneous preterm delivery more advantageous for the fetus? (Owen et al). 1990;163:868-72

A new tocolytic agent: development of an oxytocin antagonist for inhibiting uterine contractions (Wilson et al). 1990;163:195-202

Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length (Andersen et al). 1990;163:859-67

Preterm delivery: a risk factor for retained placenta (Romero et al). 1990;163:823-5

A prospective randomized comparison of oral terbutaline and magnesium oxide for the maintenance of tocolysis (Ridgway et al). 1990;163:879-82

Randomized investigation of magnesium sulfate for prevention of preterm birth (Cox et al). 1990;163:767-72

Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study (Harger et al). 1990;163:130-7

Secretion of phospholipases by bacterial pathogens may initiate preterm labor (Bennett et al). 1990;163:241-2 (Letter)

Labor, premature, animal

Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist (Wilson et al). 1990;163:1875-82

A rabbit model for bacteria-induced preterm pregnancy loss (Dombroski et al). 1990;163:1938-43

Labor complications

Effect of uterine contractility and maternal hypotension on prolonged decelerations after bupivacaine epidural anesthesia (Steiger and Nageotte). 1990;163:808-12

Uterine hyperstimulation after low-dose prostaglandin E_2 therapy: tocolytic treatment in 181 cases (Egarter et al). 1990;163:794-6

Labor stage, third

Preterm delivery: a risk factor for retained placenta (Romero et al). 1990;163:823-5

Laboratory diagnosis; *see* **Diagnosis, laboratory**

Laparoscopy; *see* **Peritoneoscopy**

Laparotomy

Management of selected cystic adnexal masses in postmenopausal women by operative laparoscopy: a pilot study (Parker and Berek). 1990;163:1574-7

Larynx, abnormalities

Prenatal diagnosis of laryngeal atresia (Watson et al). 1990;163:1456-7

Laser surgery

Carbon dioxide laser energy disperses human papillomavirus deoxyribonucleic acid onto treatment fields (Ferenczy et al). 1990;163:1271-4

Fluid cooling of artificial sapphire tips of laser (Loffer) (Letter); (Baggish) (Reply). 1990;163:681

Sapphire probes in obstetric laser surgery (Corson) (Letter); (Baggish and Daniell) (Reply). 1990;163:246-7

Lectins

Lectins and heparin-binding features of human spermatozoa as analyzed by flow cytometry (Vasquez et al). 1990;163:2006-12

Leiomyoma

Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81

Efficacy and safety considerations in women with uterine leiomyomas treated with gonadotropin-releasing hormone agonists: the estrogen threshold hypothesis (Friedman et al). 1990;163:1114-9 (Clin. opinion)

Malignant leiomyomatosis peritonealis disseminata (Akkersdijk et al). 1990;163:591-3

Preferential mitogenic activity for myoblast-like cells can be extracted from uterine leiomyoma tissues (Koutsilieris et al). 1990;163:1665-70

Leiomyomatosis; *see* **Leiomyoma**

Leiomyosarcoma

Malignant leiomyomatosis peritonealis disseminata (Akkersdijk et al). 1990;163:591-3

Letters

Letters. 1990;163:240-71, 677-712, 1089-107, 1364-77, 1713-7, 2024-30

Leukocytes

Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells (Dudley et al). 1990;163:1890-3

Leuprolide

Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis (Sakata et al). 1990;163:1679-84

Levonorgestrel

Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)

Levonorgestrel—cont'd
Effects of three combined oral contraceptive preparations containing desogestrel plus ethinyl estradiol on lipid metabolism in comparison with two levonorgestrel preparations (Kloosterboer and Rekers). 1990;163:370-3 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Pharmacokinetics of gestagens: some problems (Fotherby). 1990;163:323-8 (Supplement)
Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women (Kuhnz). 1990;163:2120-7 (Symposium)
LH
Antiprogestin treatment decreases midluteal luteinizing hormone pulse amplitude and primarily exerts a pituitary inhibition (Shoupe et al). 1990;163:1982-5
The GnRH pulse generator (Knobil). 1990;163:1721-6 (Supplement)
Gonadotropin-releasing hormone deficiency in men: diagnosis and treatment with exogenous gonadotropin-releasing hormone (Crowley and Whitcomb). 1990;163:1752-8 (Supplement)
The luteinizing hormone–releasing hormone pulse generator in men: abnormalities and clinical management (Matsumoto et al). 1990;163:1743-51 (Supplement)
Nature of gonadotropin-releasing hormone self-priming of luteinizing hormone secretion during the normal menstrual cycle (Sollenberger et al). 1990;163:1529-34
Ovulation prediction from cyclic changes in salivary electrical conductivity (Loewit et al). 1990;163:708-10 (Letter)
LH-FSH releasing hormone
The luteinizing hormone–releasing hormone pulse generator in men: abnormalities and clinical management (Matsumoto et al). 1990;163:1743-51 (Supplement)
Lichen sclerosis; *see* **Vulvar diseases**
Lidocaine
Effectiveness of 1% lidocaine dorsal penile nerve block in infant circumcision (Arnett et al). 1990;163:1074-80
Life tables
Survival rates of monoamniotic twins do not decrease after 30 weeks' gestation (Carr et al). 1990;163:719-22
Lipids
The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction (Hoppe). 1990;163:388-91 (Supplement)
Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: results of a controlled, randomized, prospective study (Kjos et al). 1990;163:1822-7
Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom Study (Simon et al). 1990;163:382-7 (Supplement)
Effects of oral contraceptives on lipid metabolism (Gevers Leuven et al). 1990;163:1410-3 (Supplement)
Effects of three combined oral contraceptive preparations containing desogestrel plus ethinyl estradiol on lipid metabolism in comparison with two levonorgestrel preparations (Kloosterboer and Rekers). 1990;163:370-3 (Supplement)
Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women (Wild et al). 1990;163:1998-2005
Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease (Juhan-Vague and Vague). 1990;163:313-5 (Supplement)
Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level (Skouby et al). 1990;163:343-8 (Supplement)
Metabolic changes during the normal menstrual cycle: a longitudinal study (Lebech et al). 1990;163:414-6 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Oral contraceptives in risk groups: Exclusion or monitoring? (Guillebaud). 1990;163:443-6 (Supplement)
Some aspects of the relationship between oral contraceptives, lipid abnormalities, and cardiovascular disease (Samsioe and Mattsson). 1990;163:354-8 (Supplement)
Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives (Kuhl et al). 1990;163:363-9 (Supplement)
Lipogenesis; *see* **Lipids**
Lipoproteins
The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction (Hoppe). 1990;163:388-91 (Supplement)
Estrogenic effect of gestodene- or desogestrel-containing oral contraceptives on lipoprotein metabolism (Gevers Leuven et al). 1990;163:358-62 (Supplement)
Oral contraceptives, lipoproteins, and atherosclerosis (Adams et al). 1990;163:1388-92 (Supplement)
Role of plasma lipoproteins in the pathogenesis of atherosclerotic disease, with special reference to sex hormone effects (Tikkanen). 1990;163:296-304 (Supplement)
Some aspects of the relationship between oral contraceptives, lipid abnormalities, and cardiovascular disease (Samsioe and Mattsson). 1990;163:354-8 (Supplement)
Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives (Kuhl et al). 1990;163:363-9 (Supplement)
Lipoproteins, HDL
Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women (Wild et al). 1990;163:1998-2005
Lipoproteins, HDL cholesterol
The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction (Hoppe). 1990;163:388-91 (Supplement)
Lipoproteins, LDL cholesterol
The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction (Hoppe). 1990;163:388-91 (Supplement)
Listeria
Intrauterine *Listeria* infection: prenatal diagnosis by biophysical assessment and amniocentesis (Liner). 1990;163:1596-7
Liver
Liver histopathologic findings in women with sickle cell disease given prophylactic transfusion during pregnancy (Yeomans et al). 1990;163:958-64
Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors (Devaskar et al). 1990;163:1350-6
Studies on the mechanism of action of oral contraceptives with regard to fibrinolytic variables (Kooistra et al). 1990;163:404-13 (Supplement)
Liver diseases
Endometrioma of the liver (Rovati et al). 1990;163:1490-2

Oral contraception in disease states (Breckwoldt et al). 1990;163:2213-6 (Symposium)
Recurrent acute fatty liver of pregnancy (Barton et al). 1990;163:534-8
Longitudinal studies
Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia (August et al). 1990;163:1612-21
Metabolic changes during the normal menstrual cycle: a longitudinal study (Lebech et al). 1990;163:414-6 (Supplement)
Low birth weight infant; *see* **Infant, low birth weight**
Lung
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66
Lung, animal
Dose response of thyrotropin-releasing hormone on pulmonary maturation in corticosteroid-treated preterm rabbits (Tabor et al). 1990;163:669-76
Effects of clonidine on breathing movements and electrocortical activity in the fetal lamb (Bamford et al). 1990;163:661-8
Lupus anticoagulant
Laboratory identification of the lupus anticoagulant in normal pregnancy and pregnancy-induced hypertension (Riveros et al) (Letter); (Lockwood) (Reply). 1990;163:704-5
Luteal phase
Luteal insufficiency: correlation between endometrial dating and integrated progesterone output in clomiphene citrate–induced cycles (Hecht et al). 1990; 163:1986-91
Luteinizing hormone; *see* **LH**
Lutrepulse; *see* **Pituitary hormone releasing hormones, analogs and derivatives**
Lymph nodes
The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes (Lewandowski et al). 1990;163:1333-7
Lymphangioma
Spontaneous resolution of a nuchal fetal cystic hygroma diagnosed early in the second trimester of pregnancy (Meizner et al) (Letter); (Bronshstein and Blumenfeld) (Reply). 1990;163:267-9
Lymphocyte stimulation; *see* **Lymphocyte transformation**
Lymphocyte transformation
Lymphocyte proliferative response to fertilization antigen in patients with antisperm antibodies (Naz et al). 1990;163:610-3
Lymphokines
Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells (Dudley et al). 1990;163:1890-3

M

Macaca fascicularis
Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys (Jayo et al). 1990;163:614-8
Macaca mulatta
Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle (Figueroa et al). 1990;163:648-54
Macrosomia
Infants of diabetic mothers with accelerated fetal growth by ultrasonography: Are they all alike? (Keller et al). 1990;163:893-7
Magnesium
Effect of tocolytic agents on fetal umbilical velocimetry (Wright et al). 1990;163:748-50
Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy (Skajaa et al). 1990;163:1323-33
Magnesium supplementation in pregnancy (Spätling and Fallenstein) (Letter); (Sibai) (Reply). 1990;163:240-1
Magnesium oxide
A prospective randomized comparison of oral terbutaline and magnesium oxide for the maintenance of tocolysis (Ridgway et al). 1990;163:879-82
Magnesium sulfate
Adjusting the loading dose of magnesium sulfate for tocolysis (Wright et al). 1990;163:889-92
Combination therapy has potential complications (Frohlich) (Letter); (Snyder and Cardwell) (Reply). 1990;163: 244
Magnesium sulfate and promethazine do not interact to cause hypotension in gravid ewes (Pollack et al). 1990;163:787-94
Magnesium sulfate is not an ideal anticonvulsant (Goodlin) (Letter); (Sibai) (Reply). 1990;163:1714-5
Neuroophthalmologic effects of intravenous magnesium sulfate (Digre et al). 1990;163:1848-52
Nifedipine enhances the cardiac toxicity of magnesium sulfate in the isolated perfused Sprague-Dawley rat heart (Thorp et al). 1990;163:655-6
Phenytoin and magnesium sulfate in eclampsia (Dommisse) (Letter); (Ryan) (Reply). 1990;163:1089-91
Randomized investigation of magnesium sulfate for prevention of preterm birth (Cox et al). 1990;163: 767-72
Magnetic resonance imaging
Pelvimetry by magnetic resonance imaging in breech presentation (van Loon et al). 1990;163:1256-60
Prenatal diagnosis of fetal intracranial calcifications (Koga et al). 1990;163:1543-5
Study of internal structure of the human fetus in utero by echo-planar magnetic resonance imaging (Johnson et al). 1990;163:601-7
Major histocompatibility complex
Sharing of human leukocyte antigens in primary and secondary recurrent spontaneous abortions (Ho et al). 1990;163:178-88
Mammography
Breast disease programs and mammography training (Dodd and McLelland) (Letter); (Gleicher) (Reply). 1990;163:689-91
Martin-Bell syndrome
Cytogenetic uncertainties surrounding the fragile X in Martin-Bell syndrome (Sindwani and Verma). 1990;163:1713-4 (Letter)
Marvelon; *see* **Desogestrel/ethinyl estradiol**
Masculinization; *see* **Sex characteristics**
Mass screening
Intrapartum hepatitis B screening in a low-risk population (Ernest et al). 1990;163:978-80
The perinatal impact of cocaine, amphetamine, and opiate

Mass screening—cont'd
use detected by universal intrapartum screening (Gillogley et al). 1990;163:1535-42
Maternal alcohol abuse; *see* **Alcoholism**
Maternal-fetal exchange
The effect of maternal hyperoxia on behavioral activity in growth-retarded human fetuses (Gagnon et al). 1990;163:1894-9
Metabolism of cocaine by human placentas: implications for fetal exposure (Roe et al). 1990;163:715-8
Placental transfer of cefazolin and piperacillin in pregnancies remote from term complicated by Rh isoimmunization (Brown et al). 1990;163:938-43
Maternal-fetal exchange, animal
Transplacental transfer of zidovudine in the near-term pregnant baboon (Hankins et al). 1990;163:728-32
Maternal metabolism; *see* **Metabolism**
Maternal mortality
Can anesthetic-related maternal mortality be reduced? (Conklin). 1990;163:253-4 (Letter)
A chill wind blows: Webster, obstetrics, and the health of women (Chavkin and Rosenfield). 1990;163:450-2 (Clin. opinion)
Cocaine-related maternal death (Burkett et al). 1990;163:40-1
Maternal outcome; *see* **Pregnancy outcome**
Maternal serum α-fetoproteins; *see* **Alpha fetoproteins**
Matrix glycoprotein receptors; *see* **Receptors, glycoprotein**
Maylard incision
The Maylard incision in gynecologic cancer (Helmkamp and Krebs). 1990;163:1554-7
Mean arterial blood pressure; *see* **Blood pressure**
Meconium aspiration
Amnioinfusion and prevention of meconium aspiration syndrome (Falciglia) (Letter); (Sadovsky and Amon) (Reply). 1990;163:258-9
Is meconium aspiration an intrauterine event? (Falciglia) (Letter); (Kalhan et al) (Reply). 1990;163:250-1
Medical education; *see* **Education, medical**
Medical ethics; *see* **Ethics**
Medical residency; *see* **Internship and residency**
Medroxyprogesterone
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163:1932-8
Membrane glycoproteins
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84
Meningomyelocele
Diagnosis and management of prenatally detected myelomeningocele: a preliminary report (Hogge et al). 1990;163:1061-5
Menopause
Endometrial assessment by vaginal ultrasonography before endometrial sampling in patients with postmenopausal bleeding (Goldstein et al). 1990;163:119-23
Management of selected cystic adnexal masses in postmenopausal women by operative laparoscopy: a pilot study (Parker and Berek). 1990;163:1574-7
Relationship between plasma calcium fractions, other bone-related variables, and serum follicle-stimulating hormone levels in premenopausal, perimenopausal, and postmenopausal women (Nordin et al). 1990;163:140-5
Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women (Cassidenti et al). 1990;163:1953-60
Menopause, premature, animal
Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys (Jayo et al). 1990;163:614-8
Menorrhagia
Early hypothyroidism in patients with menorrhagia (Blesing and Hambley) (Letter); (Wilansky and Greisman) (Reply). 1990;163:697
Screening infertile women for subclinical hypothyroidism (Strickland et al) (Letter); (Wilansky and Greisman) (Reply). 1990;163:262-3
Menstrual cycle
Changes in platelet angiotensin II binding sites associated with the human menstrual cycle (Baker et al). 1990;163:175-6
Luteal insufficiency: correlation between endometrial dating and integrated progesterone output in clomiphene citrate–induced cycles (Hecht et al). 1990;163:1986-91
Metabolic changes during the normal menstrual cycle: a longitudinal study (Lebech et al). 1990;163:414-6 (Supplement)
Nature of gonadotropin-releasing hormone self-priming of luteinizing hormone secretion during the normal menstrual cycle (Sollenberger et al). 1990;163:1529-34
Mental retardation
Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33
Mesenteric arteries, animal
Role of the endothelium in adrenergic responses of mesenteric artery rings of pregnant rats (Parent et al). 1990;163:229-34
Mestranol
Pharmacokinetics of ethinyl estradiol and mestranol (Goldzieher and Brody). 1990;163:2114-9 (Symposium)
Metabolic diseases, complications
Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease (Juhan-Vague and Vague). 1990;163:313-5 (Supplement)
Metabolism
Correlations between antepartum maternal metabolism and newborn behavior (Rizzo et al). 1990;163:1458-64
Metalloproteinases, antagonists or inhibitors
Changes in active and latent collagenase in human placenta around the time of parturition (Rajabi et al). 1990;163:499-503
Metastasis; *see* **Neoplasm metastasis**
Methotrexate
Treatment of cervical pregnancy with methotrexate (Sherer) (Letter); (Palti) (Reply). 1990;163:693-4
Methoxyverapamil; *see* **Verapamil**
Mice
Ovarian granulosa cell tumorigenesis in SWR-derived F1 hybrid mice: preneoplastic follicular abnormality and malignant disease progression (Tennent et al). 1990;163:625-34
Microspheres
Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb (Paulick et al). 1990;163:1357-63
Microsurgery
Adhesion formation after ovarian wound repair in New

Zealand white rabbits: a comparison of ovarian microsurgical closure with ovarian nonclosure (Wiskind et al). 1990;163:1674-8

Mifepristone
Antiprogestin treatment decreases midluteal luteinizing hormone pulse amplitude and primarily exerts a pituitary inhibition (Shoupe et al). 1990;163:1982-5
In vitro effect of RU 486 on sperm-egg interaction in mice (Juneja and Dodson). 1990;163:216-21
Induction of labor with mifepristone (RU 486) in intrauterine fetal death (Cabrol et al). 1990;163:540-2
Mifepristone (RU 486) failure in an ovarian heterotopic pregnancy (Levin et al). 1990;163:543-4

Migraine
Oral contraceptives in risk groups: Exclusion or monitoring? (Guillebaud). 1990;163:443-6 (Supplement)

Milk, human
Distribution of CA 125 in embryonic tissues and adult derivatives of the fetal periderm (Hardardottir et al). 1990;163:1925-31

Mitochondria
Ultrastructural aspects of preeclampsia. II. Mitochondrial changes (Shanklin and Sibai). 1990;163:943-53

Mitogens, endogenous; *see* **Growth substances**

Mitral valve, surgery
Effect of mitral valve prosthetic surgery on the outcome of a growth-retarded fetus: a case report (Izquierdo et al). 1990;163:584-7

Mitral valve stenosis
β-Adrenergic receptor blockade in the management of pregnant women with mitral stenosis (Al Kasab et al). 1990;163:37-40

Mole, hydatidiform; *see* **Hydatidiform mole**

Molybdenum, deficiency
Antenatal diagnosis of molybdenum cofactor deficiency (George et al). 1990;163:1203-4

Moniliasis, vulvovaginal
Bacterial vaginosis and trichomoniasis vaginitis are risk factors for cuff cellulitis after abdominal hysterectomy (Soper et al). 1990;163:1016-23

Monkeys, cynomolgus; *see* ***Macaca fascicularis***

Monkeys, rhesus; *see* ***Macaca mulatta***

Monoamniotic twins; *see* **Twins, monozygotic**

Monoclonal antibodies; *see* **Antibodies, monoclonal**

Morbidity
The differential neonatal morbidity of the intrauterine growth retardation syndrome (Villar et al). 1990;163:151-7
Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986 (Saftlas et al). 1990;163:460-5
Perinatal survival with expectant management of midtrimester rupture of membranes (Major and Kitzmiller). 1990;163:838-44

Mortality; *see* **Infant mortality**; **Maternal mortality**

Multiple drug resistance; *see* **Drug resistance**

Multiple endocrine adenomatosis; *see* **Neoplasms, multiple endocrine**

Multiple pregnancy; *see* **Pregnancy, multiple**

Muscle, smooth
Gestational changes in mechanical properties of skinned muscle tissues of human myometrium (Izumi et al). 1990;163:638-47

Mycoplasma infections
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91

Myelomeningocele; *see* **Meningomyelocele**

Myoblastoma
Preferential mitogenic activity for myoblast-like cells can be extracted from uterine leiomyoma tissues (Koutsilieris et al). 1990;163:1665-70

Myocardial infarction
Oral contraceptives and myocardial infarction (Thorneycroft). 1990;163:1393-7 (Supplement)
Past use of oral contraceptives and cardiovascular disease: a meta-analysis in the context of the Nurses' Health Study (Stampfer et al). 1990;163:285-91 (Supplement)

Myoma
Studies of the spontaneous motility and the effect of histamine on isolated myometrial strips of the nonpregnant human uterus: the influence of various uterine abnormalities (Martinez-Mir et al). 1990;163:189-95

Myometrium
Gestational changes in mechanical properties of skinned muscle tissues of human myometrium (Izumi et al). 1990;163:638-47
Studies of the spontaneous motility and the effect of histamine on isolated myometrial strips of the nonpregnant human uterus: the influence of various uterine abnormalities (Martinez-Mir et al). 1990;163:189-95

Myometrium, animal
Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle (Figueroa et al). 1990;163:648-54

N

Naloxone
Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women (Jaffe et al). 1990;163:1727-31 (Supplement)

Narcotics; *see also specific drugs*
The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening (Gillogley et al). 1990;163:1535-42

Natriuretic peptides, atrial
Interrelationship between atrial natriuretic factor concentrations and acute volume expansion in pregnant and nonpregnant women (Hatjis et al). 1990;163:45-50
Plasma atrial arginine vasopressin levels vary little between preeclamptic and control patients (Elias and Varizi). 1990;163:245 (Letter)

Necrotizing fasciitis; *see* **Fasciitis**

Negroid race
The effect of race on the relationship between fetal death and altered fetal growth (Ferguson and Myers). 1990;163:1222-30

Neonatal intensive care; *see* **Intensive care, neonatal**

Neonatal mortality; *see* **Infant mortality**

Neonatal outcome; *see* **Pregnancy outcome**

Neonatal screening
Cost-effectiveness of intrapartum screening and treatment for maternal group B streptococci colonization (Strickland et al). 1990;163:4-8 (Clin. opinion)

Neonatal sepsis; *see* **Infant, newborn, diseases**

Neoplasm invasiveness
Invasive carcinoma of the vulva (Cavanagh et al). 1990;163:1007-15

Neoplasm metastasis
The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes (Lewandowski et al). 1990;163:1333-7
Neoplasms, multiple endocrine
Multiple endocrine adenomatosis type I in pregnancy (Smythe et al). 1990;163:1037-9
Nephrogenic diabetes insipidus; *see* **Diabetes insipidus**
Nerve block
Effectiveness of 1% lidocaine dorsal penile nerve block in infant circumcision (Arnett et al). 1990;163:1074-80
Neuromuscular blocking agents
Differential sensitivities of fetal muscle groups to *d*-tubocurarine (Szeto and Hinman). 1990;163:202-9
Neuroophthalmologic effects; *see* **Eye, physiopathology**
Newborn diseases; *see* **Infant, newborn, diseases**
Niemann-Pick disease
Niemann-Pick disease associated with nonimmune hydrops fetalis (Meizner et al). 1990;163:128-9
Nifedipine
Combination therapy has potential complications (Frohlich) (Letter); (Snyder and Cardwell) (Reply). 1990;163:244
A comparison of tocolysis with nifedipine or ritodrine: analysis of efficacy and maternal, fetal, and neonatal outcome (Ferguson et al). 1990;163:105-11
The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term (Moretti et al). 1990;163:1844-8
Nifedipine enhances the cardiac toxicity of magnesium sulfate in the isolated perfused Sprague-Dawley rat heart (Thorp et al). 1990;163:655-6
Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy (Isla and Dyer). 1990;163:1337-44
Nitroprusside
The effect of adrenocorticotropic hormone infusion on subsequent pituitary response in the sheep fetus (Cuthrell et al). 1990;163:170-4
Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy (Isla and Dyer). 1990;163:1337-44
Nonimmune hydrops; *see* **Edema**
Nonstress tests; *see* **Fetal monitoring**
Nonsuppressible insulin-like activity
Insulin-like growth factor II and nonsuppressible insulin-like activity levels in newborns (Samaan et al). 1990;163:1836-9
Norepinephrine
Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes (Plessinger and Woods). 1990;163:1659-64
Norethindrone
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163:1932-8
Norgestimate
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Gastrointestinal metabolism of contraceptive steroids (Back et al). 1990;163:2138-45 (Symposium)
Pharmacologic and pharmacokinetic characteristics of norgestimate and its metabolites (McGuire et al). 1990;163:2127-31 (Symposium)
Nuchal cystic hygroma; *see* **Lymphangioma**
Nucleotide mapping
Differential diagnosis between complete mole and hydropic abortus by deoxyribonucleic acid fingerprints (Nobunaga et al). 1990;163:634-8
Discrepancy in the diagnoses of hydatidiform mole by macroscopic and microscopic findings and the deoxyribonucleic acid fingerprint method (Takahashi et al). 1990;163:112-3

O

Obesity in diabetes
Does fasting interval affect the glucose challenge test? (Berkus et al). 1990;163:1812-7
Obstetrical forceps
Risk factors for third-degree and fourth-degree perineal lacerations in forceps and vacuum deliveries (Combs et al). 1990;163:100-4
Use of midforceps applauded (Escamilla). 1990;163:2027 (Letter)
Obstetrics
A chill wind blows: Webster, obstetrics, and the health of women (Chavkin and Rosenfield). 1990;163:450-2 (Clin. opinion)
Oligohydramnios; *see* **Amniotic fluid**
Omphalocele; *see* **Hernia, umbilical**
Oocytes
Factors affecting embryo implantation after human in vitro fertilization: a hypothesis (Paulson et al). 1990;163:2020-3
Human oocyte and preembryo donation: an evolving method for the treatment of infertility (Sauer and Paulson). 1990;163:1421-4 (Clin. opinion)
Oocytes, animal
Platelet activating factor enhances in vitro fertilization of rabbit oocytes (Roudebush et al). 1990;163:1670-3
Oophorectomy; *see* **Ovariectomy**
Oophoritis
Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81
Operative laparoscopy; *see* **Laparotomy**
Opiates; *see* **Narcotics**
Opioid peptides; *see* **Endorphins**
Organ donors; *see* **Tissue donors**
Orthostatic hypotension; *see* **Hypotension, orthostatic**
Osteomyelitis
Postpartum osteomyelitis caused by group B streptococcus (Berkowitz and McCaffrey). 1990;163:1200-1
Osteoporosis
Prevalence and determinants of estrogen replacement therapy in elderly women (Cauley et al). 1990;163:1438-44
The relationship between plasma estradiol and the increase in bone density in postmenopausal women after treatment with subcutaneous hormone implants (Studd et al). 1990;163:1474-9
Osteoporosis, animal
Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys (Jayo et al). 1990;163:614-8

Outpatients
Outpatient obstetric management of women with type I diabetes (Bourgeois and Duffer). 1990;163:1065-73
Ovarian cysts
Hemoperitoneum as a result of coital injury without associated vaginal injury (McColgin et al). 1990;163:1503-5
Ovarian diseases
Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis (Nagamani and Stuart). 1990;163:1992-7
Ovarian failure; *see* **Ovary, physiopathology**
Ovarian neoplasms
Autologous antibodies eluted from membrane fragments in human ovarian epithelial neoplastic effusions. III. Cytotoxic potential in vitro and characterization of antigen(s) (Kutteh et al). 1990;163:1301-6
Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81
Expression of P-glycoprotein in epithelial ovarian cancer: evaluation as a marker of multidrug resistance (Rubin et al). 1990;163:69-73
The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemotherapy after surgical cytoreduction (Hunter et al). 1990;163:1164-7
Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies (Mutch et al). 1990;163:1920-4
Ovarian pregnancy; *see* **Pregnancy, ectopic**
Ovarian stroma, pathology; *see* **Stroma ovarii, pathology**
Ovariectomy
Parameters of hypothalamic aging (Seifer and Jones) (Letter); (Alexander and Aksel) (Reply). 1990;163:1715
Ovariectomy, animal
Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys (Jayo et al). 1990;163:614-8
Ovary, physiology
Oxytocin and vasopressin binding sites in human and bovine ovaries (Fuchs et al). 1990;163:1961-7
Ovary, physiology, animal
Oxytocin and vasopressin binding sites in human and bovine ovaries (Fuchs et al). 1990;163:1961-7
Ovary, physiopathology
Human oocyte and preembryo donation: an evolving method for the treatment of infertility (Sauer and Paulson). 1990;163:1421-4 (Clin. opinion)
Ovary, surgery, animal
Adhesion formation after ovarian wound repair in New Zealand white rabbits: a comparison of ovarian microsurgical closure with ovarian nonclosure (Wiskind et al). 1990;163:1674-8
Oviducts, mammalian; *see* **Fallopian tubes**
Ovulation
Inhibition of ovulation by low-dose monophasic contraceptive containing gestodene (Thomas and Vankrieken). 1990;163:1404-9 (Supplement)
Ovulation detection
Ovulation prediction from cyclic changes in salivary electrical conductivity (Loewit et al). 1990;163:708-10 (Letter)
Ovulation induction
Polycystic ovary syndrome: abnormalities and management with pulsatile gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs (Filicori et al). 1990;163:1737-42 (Supplement)
Ovum implantation
Factors affecting embryo implantation after human in vitro fertilization: a hypothesis (Paulson et al). 1990;163:2020-3
Tubal embryo successfully transferred in utero (Shettles). 1990;163:2026-7 (Letter)
Ovum transport
The complex nature of egg transport through the oviduct (Egarter) (Letter); (Pulkkinen and Jaakkola) (Reply). 1990;163:687-8
Oxytocin
Induction of labor with pulsatile oxytocin (Cummiskey and Dawood). 1990;163:1868-74
Oxytocin and vasopressin binding sites in human and bovine ovaries (Fuchs et al). 1990;163:1961-7
Oxytocin, antagonists and inhibitors
Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist (Wilson et al). 1990;163:1875-82
A new tocolytic agent: development of an oxytocin antagonist for inhibiting uterine contractions (Wilson et al). 1990;163:195-202

P

P-glycoprotein; *see* **Glycoproteins**
Packed red blood cells; *see* **Blood transfusion**
Pain
Laparoscopic ventrosuspensions (Yoong). 1990;163:1151-3
Pancreas transplantation
Anencephalics as organ donors (Van Assche). 1990;163:599-600
Pancuronium
Differential sensitivities of fetal muscle groups to *d*-tubocurarine (Szeto and Hinman). 1990;163:202-9
Papanicolaou test; *see* **Uterus**
Papillary cystadenocarcinoma; *see* **Cystadenoma**
Papillomaviruses
Carbon dioxide laser energy disperses human papillomavirus deoxyribonucleic acid onto treatment fields (Ferenczy et al). 1990;163:1271-4
5-Fluorouracil exposure during the period of conception: report on two cases (Odom et al). 1990;163:76-7
The use of in situ hybridization to show human papillomavirus deoxyribonucleic acid in metastatic cancer cells within lymph nodes (Lewandowski et al). 1990;163:1333-7
Parity
Definition of primparas (Gates). 1990;163:677 (Letter)
Partial thromboplastin time
Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84
Influence of oral contraceptives on coagulation tests in native blood and plasma (Kunz et al). 1990;163:417-20 (Supplement)
Parturition; *see* **Labor**
Parvovirus infections
Management and outcomes of pregnancies complicated by

Parvovirus infections—cont'd
human B19 parvovirus infection: a prospective study (Rodis et al). 1990;163:1168-71
Pelvic infection; *see* **Adnexitis**
Pelvic inflammatory disease; *see* **Adnexitis**
Pelvic tuberculosis; *see* **Tuberculosis, female genital**
Pelvimetry
Pelvimetry by magnetic resonance imaging in breech presentation (van Loon et al). 1990;163:1256-60
Vaginal breech delivery: a five-year prospective evaluation of a protocol using computed tomographic pelvimetry (Christian et al). 1990;163:848-55
Pemphigus
Pemphigus in pregnancy (Metzker and Merlob) (Letter); (Kanwar and Kaur) (Reply). 1990;163:1097
Penicillamine, adverse effects
Pregnancy in a patient with treated Wilson's disease: a case report (Dupont et al). 1990;163:1527-8
Penile nerve block; *see* **Nerve block**
Pentamidine
Drug prophylaxis for human immunodeficiency virus–infected pregnant women: ethical considerations (Minkoff and Moreno). 1990;163:1111-4 (Clin. opinion)
Embryofetal effects of pentamidine isethionate administered to pregnant Sprague-Dawley rats (Harstad et al). 1990;163:912-6
Pereyra procedure; *see* **Suspensions**
Periderm; *see* **Fetal development**
Perinatal mortality; *see* **Infant mortality**
Perinatal outcome; *see* **Pregnancy outcome**
Perineum, injuries
Risk factors for third-degree and fourth-degree perineal lacerations in forceps and vacuum deliveries (Combs et al). 1990;163:100-4
Peritoneoscopy
Laparoscopic diagnosis and treatment of interstitial ectopic pregnancy: a case report (Pasic and Wolfe). 1990;163:587-8
Laparoscopic ventrosuspensions (Yoong). 1990;163:1151-3
Mifepristone (RU 486) failure in an ovarian heterotopic pregnancy (Levin et al). 1990;163:543-4
Modified laparoscopy for monitoring transcervical surgery (Lin and Iwata). 1990;163:243-4 (Letter)
Peritoneum
Malignant leiomyomatosis peritonealis disseminata (Akkersdijk et al). 1990;163:591-3
Pessaries
A vesicovaginal fistula and intravesical foreign body: a rare case of the neglected pessary (Goldstein et al). 1990;163:589-91
pH; *see* **Hydrogen-ion concentration**
Pharmacokinetics
Influence of oral contraceptives on drug therapy (Teichmann). 1990;163:2208-13 (Symposium)
Interactions with oral contraceptives (Fotherby). 1990;163:2153-9 (Symposium)
Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: factors influencing steroid metabolism (Jung-Hoffmann and Kuhl). 1990;163:2183-97 (Symposium)
Pharmacokinetics of ethinyl estradiol and mestranol (Goldzieher and Brody). 1990;163:2114-9 (Symposium)
Pharmacokinetics of gestagens: some problems (Fotherby). 1990;163:323-8 (Supplement)
Pharmacokinetics of gestodene and ethinyl estradiol after oral administration of a monophasic contraceptive (Täuber et al). 1990;163:1414-20 (Supplement)
Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women (Kuhnz). 1990;163:2120-7 (Symposium)
Pharmacologic and pharmacokinetic characteristics of norgestimate and its metabolites (McGuire et al). 1990;163:2127-31 (Symposium)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Selected aspects of the pharmacokinetics and metabolism of ethinyl estrogens and their clinical implications (Goldzieher). 1990;163:318-22 (Supplement)
Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin (Bergink et al). 1990;163:2132-7 (Symposium)
Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women (Cassidenti et al). 1990;163:1953-60
Why do we need pharmacokinetic studies? (Hvidberg). 1990;163:316-8 (Supplement)
Phenotype
Correlation of the testicular determinant factor sequence zinc finger Y with varying gonadal phenotypes in a series of 13 subjects with gonadal dysgenesis because of Y aneuploidy (Tho et al). 1990;163:1968-75
Phenytoin
Phenytoin and magnesium sulfate in eclampsia (Dommisse) (Letter); (Ryan) (Reply). 1990;163:1089-91
Phosphatidylglycerols
Absence of evidence of pulmonary maturity at amniocentesis in term infants of diabetic mothers (Ojomo and Coustan). 1990;163:954-7
Phosphatidylserines
Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84
Phospholipids
Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84
Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss (Branch et al). 1990;163:210-6
Inhibitory cofactor of antiphospholipid antibody in normal pregnancy sera (Cheng). 1990;163:701 (Letter)
Recurrent adverse pregnancy outcome and antiphospholipid antibodies (Reece et al). 1990;163:162-9 (Curr. dev.)
Secretion of phospholipases by bacterial pathogens may initiate preterm labor (Bennett et al). 1990;163:241-2 (Letter)
Piperacillin
Placental transfer of cefazolin and piperacillin in pregnancies remote from term complicated by Rh isoimmunization (Brown et al). 1990;163:938-43

Pituitary-adrenal system, animal
The effect of adrenocorticotropic hormone infusion on subsequent pituitary response in the sheep fetus (Cuthrell et al). 1990;163:170-4
Pituitary gland
Antiprogestin treatment decreases midluteal luteinizing hormone pulse amplitude and primarily exerts a pituitary inhibition (Shoupe et al). 1990;163:1982-5
Pituitary gland, animal
The effect of adrenocorticotropic hormone infusion on subsequent pituitary response in the sheep fetus (Cuthrell et al). 1990;163:170-4
Pituitary hormone release inhibiting hormones
Efficacy and safety considerations in women with uterine leiomyomas treated with gonadotropin-releasing hormone agonists: the estrogen threshold hypothesis (Friedman et al). 1990;163:1114-9 (Clin. opinion)
Pituitary hormone releasing hormones
The effect of large uterine fibroids on urinary bladder function symptoms (Langer et al). 1990;163:1139-41
Efficacy and safety of intravenous pulsatile gonadotropin-releasing hormone: Lutrepulse for injection (Santoro). 1990;163:1759-64 (Supplement)
The GnRH pulse generator (Knobil). 1990;163:1721-6 (Supplement)
Gonadotropin-releasing hormone deficiency in men: diagnosis and treatment with exogenous gonadotropin-releasing hormone (Crowley and Whitcomb). 1990;163:1752-8 (Supplement)
Hypothalamic amenorrhea: clinical perspectives, pathophysiology, and management (Liu). 1990;163:1732-6 (Supplement)
The luteinizing hormone–releasing hormone pulse generator in men: abnormalities and clinical management (Matsumoto et al). 1990;163:1743-51 (Supplement)
Nature of gonadotropin-releasing hormone self-priming of luteinizing hormone secretion during the normal menstrual cycle (Sollenberger et al). 1990;163:1529-34
Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women (Jaffe et al). 1990;163:1727-31 (Supplement)
Polycystic ovary syndrome: abnormalities and management with pulsatile gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs (Filicori et al). 1990;163:1737-42 (Supplement)
Practical aspects of pulsatile gonadotropin-releasing hormone administration (Schriock). 1990;163:1765-70 (Supplement)
Pulsatile GnRH in clinical medicine: introductory remarks (Jaffe and Yen). 1990;163:1719-20 (Supplement)
Pituitary hormone releasing hormones, analogs and derivatives
Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis (Sakata et al). 1990;163:1679-84
Efficacy and safety of intravenous pulsatile gonadotropin-releasing hormone: Lutrepulse for injection (Santoro). 1990;163:1759-64 (Supplement)
Polycystic ovary syndrome: abnormalities and management with pulsatile gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs (Filicori et al). 1990;163:1737-42 (Supplement)
Practical aspects of pulsatile gonadotropin-releasing hormone administration (Schriock). 1990;163:1765-70 (Supplement)
Placenta
Changes in active and latent collagenase in human placenta around the time of parturition (Rajabi et al). 1990;163:499-503
The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term (Moretti et al). 1990;163:1844-8
Immunocytochemical distribution and localization of 15-hydroxyprostaglandin dehydrogenase in human fetal membranes, decidua, and placenta (Cheung et al). 1990;163:1445-9
Interleukin-1β, human leukocyte antigen DR1, and transforming growth factor-β expression in endometrium, placenta, and placental membranes (Kauma et al). 1990;163:1430-7
Mechanism of action of interleukin-1β in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells (Petraglia et al). 1990;163:1307-12
Metabolism of cocaine by human placentas: implications for fetal exposure (Roe et al). 1990;163:715-8
Placental histology in fetuses between 18 and 23 weeks' gestation with abnormal karyotype (Kuhlmann et al). 1990;163:1264-70
Preterm delivery: a risk factor for retained placenta (Romero et al). 1990;163:823-5
Response of placental vasculature to high glucose levels in the isolated human placental cotyledon (Roth et al). 1990;163:1828-30
Second-trimester placental biopsy for rapid fetal karyotyping (Cameron et al). 1990;163:931-4
Ultrastructural aspects of preeclampsia. II. Mitochondrial changes (Shanklin and Sibai). 1990;163:943-53
Why all placentas should be examined by a pathologist in 1990 (Salafia and Vintzileos). 1990;163:1282-93 (Curr. dev.)
Placenta, animal
Does the umbilical artery systolic/diastolic ratio reflect flow or acidosis? (Copel et al). 1990;163:751-6
Placental thromboxane and prostacyclin production in an ovine diabetic model (Dickinson et al). 1990;163:1831-5
Placenta accreta
Catastrophic complications of previous cesarean section (Chazotte and Cohen). 1990;163:738-42
Ultrasonographic evaluation of uteroplacental blood flow patterns of abnormally located and adherent placentas (Guy et al). 1990;163:723-7
Placenta diseases
The association of maternal floor infarction of the placenta with adverse perinatal outcome (Andres et al). 1990;163:935-8
Lack of specific placental abnormality associated with cocaine use (Gilbert et al). 1990;163:998-9
Spontaneous infarction of placental chorioangioma and associated regression of hydrops fetalis (Chazotte et al). 1990;163:1180-1
Ultrasonographic assessment of placental abnormalities (Jauniaux and Campbell). 1990;163:1650-8
Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level (Jauniaux et al). 1990;163:1558-60
Ultrasonographic evaluation of uteroplacental blood flow patterns of abnormally located and adherent placentas (Guy et al). 1990;163:723-7

Placenta praevia
Color flow Doppler—a useful instrument in the diagnosis of vasa previa (Harding et al). 1990;163:1566-8
Ultrasonographic evaluation of uteroplacental blood flow patterns of abnormally located and adherent placentas (Guy et al). 1990;163:723-7
Placental intervillous thrombosis; *see* **Thrombosis**
Plasma calcium; *see* **Calcium**
Plasma volume
Interrelationship between atrial natriuretic factor concentrations and acute volume expansion in pregnant and nonpregnant women (Hatjis et al). 1990;163:45-50
Plasminogen activator, tissue-type
Emergency treatment with recombinant tissue plasminogen activator of pulmonary embolism in a pregnant woman with antithrombin III deficiency (Baudo et al). 1990;163:1274-5
Enhanced generation and resolution of fibrin in women above the age of 30 years using oral contraceptives low in estrogen (Gram et al). 1990;163:438-42 (Supplement)
Platelet activating factor
Platelet activating factor enhances in vitro fertilization of rabbit oocytes (Roudebush et al). 1990;163:1670-3
Platelets; *see* **Blood platelets**
Pneumonia
Risk factors associated with the increasing prevalence of pneumonia during pregnancy (Berkowitz and LaSala). 1990;163:981-5
Polycystic ovary syndrome
Evidence of heterogeneous mechanisms in lipoprotein lipid alterations in hyperandrogenic women (Wild et al). 1990;163:1998-2005
Polycystic ovary syndrome: abnormalities and management with pulsatile gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs (Filicori et al). 1990;163:1737-42 (Supplement)
Practical aspects of pulsatile gonadotropin-releasing hormone administration (Schriock). 1990;163:1765-70 (Supplement)
Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis (Nagamani and Stuart). 1990;163:1992-7
Suppression of serum insulin level by diazoxide does not alter serum testosterone of sex hormone–binding globulin levels in healthy, nonobese women (Nestler et al). 1990;163:1243-6
Polyhydramnios; *see* **Hydramnios**
Ponderal index; *see* **Fetal development**
Postmenopausal bleeding; *see* **Menopause**
Postoperative complications, animal
Adhesion formation after ovarian wound repair in New Zealand white rabbits: a comparison of ovarian microsurgical closure with ovarian nonclosure (Wiskind et al). 1990;163:1674-8
Postpartum hemorrhage
Hyperadrenergic orthostatic intolerance as a result of postpartum blood loss (Mader et al). 1990;163:1578-9
Postpartum period; *see* **Puerperium**
Pre-eclampsia
Eclampsia. VI. Maternal-perinatal outcome in 254 consecutive cases (Sibai). 1990;163:1049-55
The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term (Moretti et al). 1990;163:1844-8
Effects of hypertension on pregnancy monitoring and results (Lenox et al). 1990;163:1173-9
Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy (Skajaa et al). 1990;163:1323-33
Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986 (Saftlas et al). 1990;163:460-5
Growth factor activity in the blood of women in whom preeclampsia develops is elevated from early pregnancy (Taylor et al). 1990;163:1839-44
Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia (August et al). 1990;163:1612-21
Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome (Brown et al). 1990;163:1853-61
Magnesium supplementation in pregnancy (Spätling and Fallenstein) (Letter); (Sibai) (Reply). 1990;163:240-1
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. 1990;163:1691-712 (Supplement)
The natural history of thrombocytopenia associated with preeclampsia (Katz et al). 1990;163:1142-3
Plasma atrial arginine vasopressin levels vary little between preeclamptic and control patients (Elias and Varizi). 1990;163:245 (Letter)
Preeclampsia in diabetic pregnancies (Garner et al). 1990;163:505-8
Preeclampsia—An endothelial cell disorder plus "something else"? (Liston and Kilpatrick) (Letter); (Roberts and Taylor) (Reply). 1990;163:1365-6
Pregnancy-induced hypertension and acute fatty liver of pregnancy: atypical presentations (Brown et al). 1990;163:1154-6
A protocol for managing severe preeclampsia in the second trimester (Sibai et al). 1990;163:733-8
Ultrastructural aspects of preeclampsia. II. Mitochondrial changes (Shanklin and Sibai). 1990;163:943-53
Zinc, preeclampsia, and γ-aminobutyric acid (Brophy). 1990;163:242-3 (Letter)
Pre-eclampsia syndromes, epidemiology
Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986 (Saftlas et al). 1990;163:460-5
Predictive value of tests
Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population (Owen et al). 1990;163:873-9
Measures of test performance and predictive values (Kadar) (Letter); (Schiff et al) (Reply). 1990;163:1374-5
Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length (Andersen et al). 1990;163:859-67
Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests (Kjos et al). 1990;163:898-903
Prednisone
Intrapartum uterine rupture and inflammatory bowel disease (Peterson and Braak). 1990;163:1247-8
Preembryo biopsy; *see* **Biopsy**
Preembryo donation; *see* **Infertility, female**
Pregnancy
Assessment of cerebral hemodynamics in pregnant women

by internal carotid artery pulsed Doppler velocimetry (Ikeda and Mori). 1990;163:494-8
Blood pressure measured telemetrically from home throughout pregnancy (Mooney et al). 1990;163:30-6
Calcium supplementation during pregnancy may reduce preterm delivery in high-risk populations (Villar and Repke). 1990;163:1124-31
The course of labor after endurance exercise during pregnancy (Clapp). 1990;163:1799-805
Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes (Roberts and Mitchell). 1990;163:1560-6
The effect of physical activity during pregnancy on preterm delivery and birth weight (Klebanoff et al). 1990;163:1450-6
Interrelationship between atrial natriuretic factor concentrations and acute volume expansion in pregnant and nonpregnant women (Hatjis et al). 1990;163:45-50
Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells (Dudley et al). 1990;163:1890-3
Magnesium supplementation in pregnancy (Spätling and Fallenstein) (Letter); (Sibai) (Reply). 1990;163:240-1
Neonatal morphometrics after endurance exercise during pregnancy (Clapp and Capeless). 1990;163:1805-11
Use of serial human chorionic gonadotropin measurements to differentiate between intrauterine and ectopic pregnancies (Kadar) (Letter); (Lindblom et al) (Reply). 1990;163:259-61

Pregnancy, abdominal

An abdominal pregnancy 10 years after treatment for pelvic tuberculosis (Durukan et al). 1990;163:594-5

Pregnancy, animal

Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle (Figueroa et al). 1990;163:648-54
Embryofetal effects of pentamidine isethionate administered to pregnant Sprague-Dawley rats (Harstad et al). 1990;163:912-6
Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist (Wilson et al). 1990;163:1875-82
Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat (Mabie et al). 1990;163:1861-7
Relationship between the ovine fetal umbilical artery blood flow waveform and umbilical vascular resistance (Irion and Clark). 1990;163:222-9
Role of the endothelium in adrenergic responses of mesenteric artery rings of pregnant rats (Parent et al). 1990;163:229-34
Transplacental transfer of zidovudine in the near-term pregnant baboon (Hankins et al). 1990;163:728-32

Pregnancy, ectopic

An abdominal pregnancy 10 years after treatment for pelvic tuberculosis (Durukan et al). 1990;163:594-5
Caution in use of new test for ectopic pregnancy (Haas) (Letter); (Lindblom et al) (Reply). 1990;163:694-5
Changes in the incidence of ectopic pregnancy in Hungary (Jakobovits) (Letter); (Mäkinen et al) (Reply). 1990;163:265-6
Conservative management of cervical pregnancy with subsequent fertility (Ayromlooi) (Letter); (Bachus) (Reply). 1990;163:1093-5
Conservative treatment of ovarian pregnancy by local prostaglandin $F_{2\alpha}$ injection (Koike et al). 1990;163:696 (Letter)
Contraception and ectopic pregnancy risk (Franks et al). 1990;163:1120-3
Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels (Kratzer and Taylor). 1990;163:1497-502
Critical comments on the treatment of tubal pregnancy with prostaglandins (Dietl and Lippert) (Letter); (Egarter and Husslein) (Reply). 1990;163:707-8
Embryo migration responsible for ectopic pregnancies (Rizk). 1990;163:1369 (Letter)
Heterotopic pregnancy associated with assisted reproductive technology (Dimitry et al). 1990;163:244-5 (Letter)
Laparoscopic diagnosis and treatment of interstitial ectopic pregnancy: a case report (Pasic and Wolfe). 1990;163:587-8
Mifepristone (RU 486) failure in an ovarian heterotopic pregnancy (Levin et al). 1990;163:543-4
Ovarian pregnancy: a report of 20 cases in one institution (Raziel et al). 1990;163:1182-5
Transabdominal and transvaginal ultrasonography in the diagnosis of ectopic pregnancy: a comparative study (Kivikoski et al). 1990;163:123-8
Treatment of cervical pregnancy with methotrexate (Sherer) (Letter); (Palti) (Reply). 1990;163:693-4
Use of serial human chorionic gonadotropin measurements to differentiate between intrauterine and ectopic pregnancies (Kadar) (Letter); (Lindblom et al) (Reply). 1990;163:259-61
Vaginal probe ultrasonography and ectopic pregnancy (Goldstein) (Letter); (Stiller) (Reply). 1990;163:1099-100

Pregnancy, multiple

Credit neonatal or obstetric care? (Weissman and Jakobi) (Letter); (Milligan) (Reply). 1990;163:1716
Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33

Pregnancy, prolonged

Observations on the cause of oligohydramnios in prolonged pregnancy (Trimmer et al). 1990;163:1900-3
Prolongation of pregnancy and survival of second twin (Goodlin) (Letter); (Feichtinger) (Reply). 1990;163:270-1

Pregnancy, prolonged, animal

A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios (Harding et al). 1990;163:1904-13

Pregnancy complications

Acromegaly first diagnosed in pregnancy: the role of bromocriptine therapy (Yap et al). 1990;163:477-8
Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84
Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes (Roberts and Mitchell). 1990;163:1560-6
The effect of adjusting maternal serum α-fetoprotein levels for maternal weight in pregnancies with fetal open spina bifida (Johnson et al). 1990;163:9-11

Pregnancy complications—cont'd
Emergency treatment with recombinant tissue plasminogen activator of pulmonary embolism in a pregnant woman with antithrombin III deficiency (Baudo et al). 1990;163:1274-5
Growth factor activity in the blood of women in whom preeclampsia develops is elevated from early pregnancy (Taylor et al). 1990;163:1839-44
Is indicated or spontaneous preterm delivery more advantageous for the fetus? (Owen et al). 1990;163:868-72
Ketoconazole treatment of Cushing's syndrome in pregnancy (Divers). 1990;163:1101 (Letter)
Lack of specific placental abnormality associated with cocaine use (Gilbert et al). 1990;163:998-9
Maternal serum α-fetoprotein level useful screening test (Lao). 1990;163:240 (Letter)
Pemphigus in pregnancy (Metzker and Merlob) (Letter); (Kanwar and Kaur) (Reply). 1990;163:1097
Pregnancy in a patient with treated Wilson's disease: a case report (Dupont et al). 1990;163:1527-8
Pregnancy-induced hypertension and acute fatty liver of pregnancy: atypical presentations (Brown et al). 1990;163:1154-6
Prevalence of use of cocaine and other substances in an obstetric population (Matera et al). 1990;163:797-801
Recurrent acute fatty liver of pregnancy (Barton et al). 1990;163:534-8
Toxicity of vancomycin given during pregnancy (Gouyon and Petion) (Letter); (Reyes and Ostrea) (Reply). 1990;163:1375-6

Pregnancy complications, cardiovascular
β-Adrenergic receptor blockade in the management of pregnant women with mitral stenosis (Al Kasab et al). 1990;163:37-40
Effect of mitral valve prosthetic surgery on the outcome of a growth-retarded fetus: a case report (Izquierdo et al). 1990;163:584-7
Effect of uterine contractility and maternal hypotension on prolonged decelerations after bupivacaine epidural anesthesia (Steiger and Nageotte). 1990;163:808-12
Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986 (Saftlas et al). 1990;163:460-5
Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive value for pregnancy-induced hypertension (Hume et al). 1990;163:826-9
Intracellular sodium levels in pregnant women with hypertension (Goodlin) (Letter); (Sowers and Zemel) (Reply). 1990;163:261-2
Laboratory identification of the lupus anticoagulant in normal pregnancy and pregnancy-induced hypertension (Riveros et al) (Letter); (Lockwood) (Reply). 1990;163:704-5
Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia (August et al). 1990;163:1612-21
Magnesium sulfate and promethazine do not interact to cause hypotension in gravid ewes (Pollack et al). 1990;163:787-94
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. 1990;163:1691-712 (Supplement)
National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy: foreword (Lenfant et al). 1990;163:1689-90 (Supplement)

Pregnancy complications, cardiovascular, animal
Fetal and maternal response to intravenous infusion of a thromboxane synthetase inhibitor (Cardin et al). 1990;163:1345-9

Pregnancy complications, hematologic
Effects of hypertension on pregnancy monitoring and results (Lenox et al). 1990;163:1173-9
HELLP does not always mean immediate HELP! (Goodlin) (Letter); (Sibai) (Reply). 1990;163:1089
Liver histopathologic findings in women with sickle cell disease given prophylactic transfusion during pregnancy (Yeomans et al). 1990;163:958-64
Low fetal risks in pregnancies associated with idiopathic thrombocytopenia purpura (Burrows and Kelton). 1990;163:1147-50
The natural history of thrombocytopenia associated with preeclampsia (Katz et al). 1990;163:1142-3
A reappraisal of the need for autologous blood donation in the obstetric patient (Andres et al). 1990;163:1551-3

Pregnancy complications, infectious
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91
Detection of congenital toxoplasmosis by chorionic villus sampling and early amniocentesis (Foulon et al). 1990;163:1511-3
Drug prophylaxis for human immunodeficiency virus–infected pregnant women: ethical considerations (Minkoff and Moreno). 1990;163:1111-4 (Clin. opinion)
False-negative syphilis screening: the prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy (Berkowitz et al). 1990;163:975-7
High infectious morbidity in pregnant women with insulin-dependent diabetes: an understated complication (Stamler et al). 1990;163:1217-21
Intrapartum hepatitis B screening in a low-risk population (Ernest et al). 1990;163:978-80
Intrauterine *Listeria* infection: prenatal diagnosis by biophysical assessment and amniocentesis (Liner). 1990;163:1596-7
Management and outcomes of pregnancies complicated by human B19 parvovirus infection: a prospective study (Rodis et al). 1990;163:1168-71
Risk factors associated with the increasing prevalence of pneumonia during pregnancy (Berkowitz and LaSala). 1990;163:981-5
Risk factors for perinatal group B streptococcal disease after amniotic fluid colonization (Silver et al). 1990;163:19-25

Pregnancy complications, neoplastic
Cervical cancer: Role for progesterone during pregnancy and contraception? (Stendahl and Rogo). 1990;163:685-6 (Letter)
Colon cancer in pregnancy with elevated maternal serum α-fetoprotein level at presentation (Gonsoulin et al). 1990;163:1172-3
Multiple endocrine adenomatosis type I in pregnancy (Smythe et al). 1990;163:1037-9
Perception of teratogenic risk by pregnant women exposed to diagnostic radiation during pregnancy (Mastroiacovo et al) (Letter); (Koren) (Reply). 1990;163:695-6
Systemic cystic angiomatosis in pregnancy: a case presentation and review of the literature (Bardeguez et al). 1990;163:42-5

Pregnancy in diabetes
Absence of evidence of pulmonary maturity at amniocen-

tesis in term infants of diabetic mothers (Ojomo and Coustan). 1990;163:954-7
Ascertainment bias in diabetic outcome data (FitzSimmons and Davidson) (Letter); (Damm and Mølsted-Pedersen) (Reply). 1990;163:682-3
Correlations between antepartum maternal metabolism and newborn behavior (Rizzo et al). 1990;163:1458-64
Does fasting interval affect the glucose challenge test? (Berkus et al). 1990;163:1812-7
Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: results of a controlled, randomized, prospective study (Kjos et al). 1990;163:1822-7
Findings not new (Abell and Beischer) (Letter); (Langer) (Reply). 1990;163:2027-8
Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months post partum (Kjos et al). 1990;163:93-8
Glucose tolerance in pregnancy: ethnic variation and influence of body habitus (Green et al). 1990;163:86-92
High infectious morbidity in pregnant women with insulin-dependent diabetes: an understated complication (Stamler et al). 1990;163:1217-21
Infants of diabetic mothers with accelerated fetal growth by ultrasonography: Are they all alike? (Keller et al). 1990;163:893-7
Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors (Devaskar et al). 1990;163:1350-6
Maternal serum α-fetoprotein levels in pregnancies complicated by diabetes: implications for screening programs (Martin et al). 1990;163:1209-16
Outpatient obstetric management of women with type I diabetes (Bourgeois and Duffer). 1990;163:1065-73
Placental thromboxane and prostacyclin production in an ovine diabetic model (Dickinson et al). 1990;163:1831-5
Preeclampsia in diabetic pregnancies (Garner et al). 1990;163:505-8
Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests (Kjos et al). 1990;163:898-903
Relationships between glucose levels and insulin secretion during a glucose challenge test (Berkus et al). 1990;163:1818-21
Response of placental vasculature to high glucose levels in the isolated human placental cotyledon (Roth et al). 1990;163:1828-30

Pregnancy loss; *see* **Abortion**; **Pregnancy outcome**

Pregnancy outcome

Antiphospholipid antibodies and recurrent pregnancy loss: correlation between the activated partial thromboplastin time and antibodies against phosphatidylserine and cardiolipin (Rote et al). 1990;163:575-84
The association of maternal floor infarction of the placenta with adverse perinatal outcome (Andres et al). 1990;163:935-8
Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91
Chronic renal disease and pregnancy outcome (Cunningham et al). 1990;163:453-9
A comparison of tocolysis with nifedipine or ritodrine: analysis of efficacy and maternal, fetal, and neonatal outcome (Ferguson et al). 1990;163:105-11
Credit neonatal or obstetric care? (Weissman and Jakobi) (Letter); (Milligan) (Reply). 1990;163:1716
The diagnostic values of concurrent nonstress testing, amniotic fluid measurement, and Doppler velocimetry in screening a general high-risk population (Devoe et al). 1990;163:1040-8
Eclampsia. VI. Maternal-perinatal outcome in 254 consecutive cases (Sibai). 1990;163:1049-55
The effect of mode of delivery on the perinatal outcome in fetuses with abdominal wall defects (Moretti et al). 1990;163:833-8
Effects of hypertension on pregnancy monitoring and results (Lenox et al). 1990;163:1173-9
Inhibitory cofactor of antiphospholipid antibody in normal pregnancy sera (Cheng). 1990;163:701 (Letter)
Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome (Brown et al). 1990;163:1853-61
Management and outcomes of pregnancies complicated by human B19 parvovirus infection: a prospective study (Rodis et al). 1990;163:1168-71
Maternal exercise and early pregnancy outcome (Suzuki and Miller). 1990;163:1103 (Letter)
Perinatal outcome of forty-nine pregnancies complicated by acardiac twinning (Moore et al). 1990;163:907-12
Perinatal survival with expectant management of midtrimester rupture of membranes (Major and Kitzmiller). 1990;163:838-44
Pregnancy in a patient with treated Wilson's disease: a case report (Dupont et al). 1990;163:1527-8
Pregnancy outcomes among mothers infected with human immunodeficiency virus and uninfected control subjects (Minkoff et al). 1990;163:1598-604
Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus (Carlson et al). 1990;163:1785-7
Recurrent adverse pregnancy outcome and antiphospholipid antibodies (Reece et al). 1990;163:162-9 (Curr. dev.)
Reducing cesarean sections at a teaching hospital (Sanchez-Ramos et al). 1990;163:1081-8
Risk factors associated with the increasing prevalence of pneumonia during pregnancy (Berkowitz and LaSala). 1990;163:981-5
The stuck twin phenomenon: Ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses (Mahony et al). 1990;163:1513-22

Pregnancy trimester, first

Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels (Kratzer and Taylor). 1990;163:1497-502
Early pregnancy failure—appropriate terminology (Goldstein). 1990;163:1093 (Letter)
Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast (Rodway et al). 1990;163:1546-51
Soluble factors produced by isolated first-trimester chorionic villi directly inhibit proliferation of T cells (Silver et al). 1990;163:1914-9
Transvaginal ultrasonographic detection of congenital anomalies in the first trimester (Cullen et al). 1990;163:466-76
Ultrasonographic dating of very early pregnancy (Cadkin and McAlpin-Cadkin). 1990;163:1103 (Letter)

Pregnancy trimester, second
Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: an accurate and rapid method for detection of fetal chromosome abnormalities (Shulman et al). 1990;163:1606-9
Intraamniotic infection in the very early phase of the second trimester (Goldstein et al). 1990;163:1261-3
Perinatal survival with expectant management of midtrimester rupture of membranes (Major and Kitzmiller). 1990;163:838-44
A protocol for managing severe preeclampsia in the second trimester (Sibai et al). 1990;163:733-8
Second-trimester placental biopsy for rapid fetal karyotyping (Cameron et al). 1990;163:931-4
Second-trimester ultrasonography: determinants of visualization of fetal anatomic structures (Catanzarite and Quirk). 1990;163:1191-5
Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21 (Ginsberg et al). 1990;163:1186-90
Pregnancy trimester, third
Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: an accurate and rapid method for detection of fetal chromosome abnormalities (Shulman et al). 1990;163:1606-9
Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist (Wilson et al). 1990;163:1875-82
Placental histology in fetuses between 18 and 23 weeks' gestation with abnormal karyotype (Kuhlmann et al). 1990;163:1264-70
Premature infant; *see* **Infant, premature**
Premature labor; *see* **Labor, premature**
Premature rupture of membranes; *see* **Fetal membranes, premature rupture**
Premenstrual syndrome
Vitamin E levels in premenstrual syndrome (Chuong et al). 1990;163:1591-5
Prenatal care
The relationship of cocaine use to syphilis and human immunodeficiency virus infections among inner city parturient women (Minkoff et al). 1990;163:521-6
Prenatal diagnosis
The associated anomalies that determine prognosis in congenital omphaloceles (Tucci and Bard). 1990;163:1646-9
Color flow Doppler—a useful instrument in the diagnosis of vasa previa (Harding et al). 1990;163:1566-8
Detection of congenital toxoplasmosis by chorionic villus sampling and early amniocentesis (Foulon et al). 1990;163:1511-3
Diagnosis and management of prenatally detected myelomeningocele: a preliminary report (Hogge et al). 1990;163:1061-5
Direct analysis of uncultured cytotrophoblastic cells from second- and third-trimester placentas: an accurate and rapid method for detection of fetal chromosome abnormalities (Shulman et al). 1990;163:1606-9
Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes (Roberts and Mitchell). 1990;163:1560-6
An evaluation of the chorionic villus sampling learning curve (Silver et al). 1990;163:917-22
In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1
Intrauterine *Listeria* infection: prenatal diagnosis by biophysical assessment and amniocentesis (Liner). 1990;163:1596-7
Potential prenatal predictions of Down syndrome: a statistical analysis (O'Brien et al). 1990;163:1796-8
Prenatal diagnosis of a chest wall mass with ultrasonography and Doppler velocimetry: a case report (Smith et al). 1990;163:567-9
Prenatal diagnosis of congenital cytomegalovirus infection: two decades later (Grose and Weiner). 1990;163:447-50 (Clin. opinion)
Prenatal diagnosis of congenital cytomegalovirus infection by virus isolation from amniotic fluid (Weiner and Grose). 1990;163:1253-5
Prenatal diagnosis of fetal intracranial calcifications (Koga et al). 1990;163:1543-5
Prenatal diagnosis of laryngeal atresia (Watson et al). 1990;163:1456-7
Prognostic indicators of the resolution of nonimmune hydrops fetalis and survival of the fetus (Carlson et al). 1990;163:1785-7
Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants (Mills et al). 1990;163:1568-74
Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femur length ratio (Marquette et al). 1990;163:1604-5
Transcervical chorionic villus sampling and amniocentesis: a comparison of reliability, culture findings, and fetal outcome (Ferguson et al). 1990;163:926-31
Transvaginal ultrasonographic detection of congenital anomalies in the first trimester (Cullen et al). 1990;163:466-76
Ultrasonographic assessment of placental abnormalities (Jauniaux and Campbell). 1990;163:1650-8
Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level (Jauniaux et al). 1990;163:1558-60
Presidential address
Presidential address. 1990;163:1000-6
Preterm labor; *see* **Labor, premature**
Primiparity; *see* **Parity**
Prior surgery; *see* **Surgery, operative**
Procollagen
Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81
Prodrugs
Prodrugs: Advantage or disadvantage? (Hammerstein). 1990;163:2198-203 (Symposium)
Professional practice
A chill wind blows: Webster, obstetrics, and the health of women (Chavkin and Rosenfield). 1990;163:450-2 (Clin. opinion)
Retrospection and prospection (McLeod). 1990;163:1000-6 (Pres. address)
Progestational hormones
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163:1932-8
Progestational hormones, antagonists and inhibitors
Antiprogestin treatment decreases midluteal luteinizing

hormone pulse amplitude and primarily exerts a pituitary inhibition (Shoupe et al). 1990;163:1982-5

Progestational hormones, synthetic
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
The clinical relevance of oral contraceptive pill–induced plasma lipid changes: facts and fiction (Hoppe). 1990;163:388-91 (Supplement)
Comparative studies of 30 μg ethinyl estradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis, and platelets (Daly and Bonnar). 1990;163:430-7 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Prodrugs: Advantage or disadvantage? (Hammerstein). 1990;163:2198-203 (Symposium)
Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin (Bergink et al). 1990;163:2132-7 (Symposium)

Progesterone
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Cervical cancer: Role for progesterone during pregnancy and contraception? (Stendahl and Rogo). 1990;163:685-6 (Letter)
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163:1932-8
Corpus luteum function in early pregnancies is primarily determined by the rate of change of human chorionic gonadotropin levels (Kratzer and Taylor). 1990;163:1497-502
Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom Study (Simon et al). 1990;163:382-7 (Supplement)
Effects on bone of surgical menopause and estrogen therapy with or without progesterone replacement in cynomolgus monkeys (Jayo et al). 1990;163:614-8
Luteal insufficiency: correlation between endometrial dating and integrated progesterone output in clomiphene citrate–induced cycles (Hecht et al). 1990;163:1986-91
Prodrugs: Advantage or disadvantage? (Hammerstein). 1990;163:2198-203 (Symposium)
Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes (Plessinger and Woods). 1990;163:1659-64
A progesterone-induced blocking factor corrects high resorption rates in mice treated with antiprogesterone (Szekeres-Bartho et al). 1990;163:1320-1
Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin (Bergink et al). 1990;163:2132-7 (Symposium)

Progestogens; *see* **Progestational hormones, synthetic**
Prohormones; *see* **Prodrugs**
Prolactin
Comparison of norethindrone and medroxyprogesterone acetate with natural progesterone and estradiol in stimulating prolactin production from cultured endometrial stromal cells (Levin et al). 1990;163:1932-8
Comparisons of glycosylated and nonglycosylated amniotic fluid prolactins in normal second- and third-trimester pregnancies (McCoshen et al). 1990;163:81-5

Prolongation of pregnancy; *see* **Pregnancy, prolonged**
Prolonged rupture of membranes; *see* **Fetal membranes, prolonged rupture**
Promethazine
Magnesium sulfate and promethazine do not interact to cause hypotension in gravid ewes (Pollack et al). 1990;163:787-94

Prostacyclins; *see* **Prostaglandins X**
Prostaglandin synthase
Immunohistochemical localization of prostaglandin H synthase in the female reproductive tract and endometriosis (Van Voorhis et al). 1990;163:57-62

Prostaglandins
Critical comments on the treatment of tubal pregnancy with prostaglandins (Dietl and Lippert) (Letter); (Egarter and Husslein) (Reply). 1990;163:707-8
Immunohistochemical localization of prostaglandin H synthase in the female reproductive tract and endometriosis (Van Voorhis et al). 1990;163:57-62
Low-dose aspirin. II. Relationship of angiotensin II pressor responses, circulating eicosanoids, and pregnancy outcome (Brown et al). 1990;163:1853-61
Morphologic study of changes of collagenous tissue in the amnion and cervix during prostaglandin-induced abortion and delivery (Manabe and Yoshida). 1990;163:52-6
Venous and hepatic vascular responses to indomethacin and prostaglandin E_1 in the fetal lamb (Paulick et al). 1990;163:1357-63

Prostaglandins E_2; *See* **Dinoprostone**
Prostaglandins $F_{2\alpha}$; *see* **Dinoprost**
Prostaglandins H
Immunohistochemical localization of prostaglandin H synthase in the female reproductive tract and endometriosis (Van Voorhis et al). 1990;163:57-62

Prostaglandins X
Placental thromboxane and prostacyclin production in an ovine diabetic model (Dickinson et al). 1990;163:1831-5

Prosthetic heart valves; *see* **Heart valve prosthesis**
Protein kinase C
Evidence of a role for protein kinase C in epidermal growth factor–induced prostaglandin E_2 synthesis in amnion cells (Kniss et al). 1990;163:1883-90

Puerperium
Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months post partum (Kjos et al). 1990;163:93-8
Postpartum coronary artery dissection followed by heart transplantation (Curiel et al). 1990;163:538-9
Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy (Esposito et al). 1990;163:572-4

Pulmonary artery, surgery
Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy (Esposito et al). 1990;163:572-4

Pulmonary edema
Ritodrine infusion and cardiomyopathy (Caritis) (Letter); (Blickstein) (Reply). 1990;163:254-6

Pulmonary embolism
Emergency treatment with recombinant tissue plasminogen activator of pulmonary embolism in a pregnant woman

Pulmonary embolism—cont'd
with antithrombin III deficiency (Baudo et al). 1990;163:1274-5
Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy (Esposito et al). 1990;163:572-4
Pulmonary surfactants
Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests (Kjos et al). 1990;163:898-903
Pulsatile oxytocin; *see* **Oxytocin**
Purpura, thrombocytopenic
Low fetal risks in pregnancies associated with idiopathic thrombocytopenia purpura (Burrows and Kelton). 1990;163:1147-50

Q

Quality assurance, health care
The impact of a quality assurance process on the frequency and confirmation rate of hysterectomy (Gambone et al). 1990;163:545-50
Questionnaires
Use of the T-ACE questions to detect risk-drinking (Elliot) (Letter); (Sokol) (Reply). 1990;163:684-5

R

Racial stocks
The effect of race on the relationship between fetal death and altered fetal growth (Ferguson and Myers). 1990;163:1222-30
Radiation effects
Hyperkalemia secondary to irradiation of packed red blood cells: possible effects with intravascular fetal transfusion (Thorp et al). 1990;163:607-9
Perception of teratogenic risk by pregnant women exposed to diagnostic radiation during pregnancy (Mastroiacovo et al) (Letter); (Koren) (Reply). 1990;163:695-6
Rapid eye movements; *see* **Sleep, REM**
Rats, inbred strains
Nifedipine enhances the cardiac toxicity of magnesium sulfate in the isolated perfused Sprague-Dawley rat heart (Thorp et al). 1990;163:655-6
Rats, Sprague-Dawley; *see* **Rats, inbred strains**
Raz procedure; *see* **Suspensions**
Receptors, adrenergic, beta
β-Adrenergic receptor blockade in the management of pregnant women with mitral stenosis (Al Kasab et al). 1990;163:37-40
Receptors, angiotensin
Changes in platelet angiotensin II binding sites associated with the human menstrual cycle (Baker et al). 1990;163:175-6
Receptors, estrogen, animal
Uterine artery estrogen receptors in the nonpregnant and pregnant guinea pig (Leiberman et al). 1990;163:1685-8
Receptors, glycoprotein
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84
Receptors, insulin
Long-term maternal-fetal exposure to high-low insulin concentrations alter liver but not brain insulin receptors (Devaskar et al). 1990;163:1350-6
Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis (Nagamani and Stuart). 1990;163:1992-7
Receptors, progesterone, animal
A progesterone-induced blocking factor corrects high resorption rates in mice treated with antiprogesterone (Szekeres-Bartho et al). 1990;163:1320-1
Receptors, steroid
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Recombinant tissue plasminogen activator; *see* **Plasminogen activator, tissue-type**
Recurrent pregnancy loss; *see* **Abortion, habitual**; **Pregnancy outcome**
Red cell alloimmunization; *see* **Erythrocytes, immunology**
Renal failure, chronic; *see* **Kidney failure, chronic**
Renin-angiotensin system
Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed preeclampsia (August et al). 1990;163:1612-21
Reproducibility of results
Transcervical chorionic villus sampling and amniocentesis: a comparison of reliability, culture findings, and fetal outcome (Ferguson et al). 1990;163:926-31
Reproduction techniques
Ethics of new reproductive technologies (Blaskiewicz) (Letter); (Macnaughton) (Reply). 1990;163:1376
Heterotopic pregnancy associated with assisted reproductive technology (Dimitry et al). 1990;163:244-5 (Letter)
Residency; *see* **Internship and residency**
Respiratory distress syndrome
Digoxin-like immunoreactive factor and respiratory distress syndrome (Goodlin). 1990;163:1091 (Letter)
Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests (Kjos et al). 1990;163:898-903
Retained placenta; *see* **Placenta**
Retropubic suspension procedures; *see* **Suspensions**
Rh isoimmunization
Amniotic fluid midtrimester delta optical density measurements at 450 nm in Rh disease (Nelson) (Letter); (Queenan) (Reply). 1990;163:710-2
A case of Rh isoimmunization: Should threatened first-trimester abortion be an indication for Rh immune globulin prophylaxis? (Dayton et al). 1990;163:63-4
Effects of intravascular fetal blood transfusion on fetal intracardiac Doppler velocity waveforms (Rizzo et al). 1990;163:1231-8
The incidence of significant fetomaternal hemorrhage in patients undergoing cesarean section (Feldman et al). 1990;163:855-8
Placental transfer of cefazolin and piperacillin in pregnancies remote from term complicated by Rh isoimmunization (Brown et al). 1990;163:938-43
Postinjection kinetics of antepartum Rh immune globulin (Witter et al). 1990;163:784-6
Rhesus monkeys; *see* ***Macaca mulatta***
Risk factors
Antenatal microbiologic and maternal risk factors associated with prematurity (McGregor et al). 1990;163:1465-73
Bacterial vaginosis and trichomoniasis vaginitis are risk factors for cuff cellulitis after abdominal hysterectomy (Soper et al). 1990;163:1016-23
The diagnostic values of concurrent nonstress testing, am-

niotic fluid measurement, and Doppler velocimetry in screening a general high-risk population (Devoe et al). 1990;163:1040-8
Evaluation of a risk scoring system as a predictor of preterm birth in an indigent population (Owen et al). 1990;163:873-9
Maternal serum α-fetoprotein level useful screening test (Lao). 1990;163:240 (Letter)
Oral contraceptives in risk groups: Exclusion or monitoring? (Guillebaud). 1990;163:443-6 (Supplement)
Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length (Andersen et al). 1990;163:859-67
Risk factors associated with the increasing prevalence of pneumonia during pregnancy (Berkowitz and LaSala). 1990;163:981-5
Risk factors for perinatal group B streptococcal disease after amniotic fluid colonization (Silver et al). 1990; 163:19-25
Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study (Harger et al). 1990;163:130-7
Risk factors for third-degree and fourth-degree perineal lacerations in forceps and vacuum deliveries (Combs et al). 1990;163:100-4
Smoking and the antioxidant ascorbic acid: plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women (Basu et al). 1990;163:1948-52
Use of the T-ACE questions to detect risk-drinking (Elliot) (Letter); (Sokol) (Reply). 1990;163:684-5

Ritodrine
Awaiting cervical change for the diagnosis of preterm labor does not compromise the efficacy of ritodrine tocolysis (Utter et al). 1990;163:882-6
A comparison of tocolysis with nifedipine or ritodrine: analysis of efficacy and maternal, fetal, and neonatal outcome (Ferguson et al). 1990;163:105-11
Effects of ritodrine tocolysis on cardiac isozymes and electrocardiography (Hendricks and Katz) (Letter); (Hadi and Albazzaz) (Reply). 1990;163:699-701
Ritodrine infusion and cardiomyopathy (Caritis) (Letter); (Blickstein) (Reply). 1990;163:254-6

Round ligament
Hemoperitoneum as a result of coital injury without associated vaginal injury (McColgin et al). 1990;163:1503-5

RU-486; *see* **Mifepristone**

Running
The course of labor after endurance exercise during pregnancy (Clapp). 1990;163:1799-805
Neonatal morphometrics after endurance exercise during pregnancy (Clapp and Capeless). 1990;163:1805-11

Rupture of uterus; *see* **Uterine rupture**

S

Sacrococcygeal region
Survival in a fetus with sacrococcygeal teratoma and hydrops (Nakayama). 1990;163:682 (Letter)

Saliva, analysis
Ovulation prediction from cyclic changes in salivary electrical conductivity (Loewit et al). 1990;163:708-10 (Letter)

Salpingectomy; *see* **Sterilization, tubal**

Salpingitis
Collagen metabolism in gynecologic patients: changes in the concentration of the aminoterminal propeptide of type III procollagen in serum (Puistola et al). 1990;163:1276-81

Sampling studies
An evaluation of the chorionic villus sampling learning curve (Silver et al). 1990;163:917-22

Sapphire probes; *see* **Surgical instruments**

Screening, genetic; *see* **Genetic screening**

Screening, mass; *see* **Mass screening**

Second trimester; *see* **Pregnancy trimester, second**

Selection bias
Ascertainment bias in diabetic outcome data (FitzSimmons and Davidson) (Letter); (Damm and Mølsted-Pedersen) (Reply). 1990;163:682-3

Self medication
Autogenic masculinization (Punch and Ansbacher). 1990;163:114-6

Semantics
A conceptual thought (Pearse). 1990;163:1098 (Letter)

Serology
False-negative syphilis screening: the prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy (Berkowitz et al). 1990;163:975-7

Serum lipids; *see* **Lipids**

Sex characteristics
Autogenic masculinization (Punch and Ansbacher). 1990;163:114-6

Sex determination
Rapid determination of fetal sex (Stephens) (Letter); (Pinckert et al) (Reply). 1990;163:683-4

Sex hormone–binding globulin
Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor (Juchem and Pollow). 1990;163:2171-83 (Symposium)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Pharmacokinetics of gestagens: some problems (Fotherby). 1990;163:323-8 (Supplement)
Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women (Kuhnz). 1990;163:2120-7 (Symposium)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Suppression of serum insulin level by diazoxide does not alter serum testosterone of sex hormone–binding globulin levels in healthy, nonobese women (Nestler et al). 1990;163:1243-6

Sex hormones; *see also* **Contraceptives, oral**
First European Conference on Sex Steroids and Metabolism: introductory remarks (Jespersen et al). 1990;163:273 (Supplement)
Human oocyte and preembryo donation: an evolving method for the treatment of infertility (Sauer and Paulson). 1990;163:1421-4 (Clin. opinion)
Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system (Beck-Nielsen et al). 1990;163:292-5 (Supplement)
Metabolic changes during treatment with two different progestogens (Refn et al). 1990;163:374-7 (Supplement)
Oral contraceptives in risk groups: Exclusion or monitoring? (Guillebaud). 1990;163:443-6 (Supplement)

Sex hormones—cont'd
Pharmacokinetics of gestagens: some problems (Fotherby). 1990;163:323-8 (Supplement)
Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)
Role of plasma lipoproteins in the pathogenesis of atherosclerotic disease, with special reference to sex hormone effects (Tikkanen). 1990;163:296-304 (Supplement)
Why do we need pharmacokinetic studies? (Hvidberg). 1990;163:316-8 (Supplement)
Sex preselection
How sperm commence movement and their isolation for in vitro fertilization and sex selection (Shettles). 1990;163:270 (Letter)
Sex steroids; *see* **Sex hormones**
Sexually transmitted diseases
Bacterial vaginosis: diagnostic and pathogenetic findings during topical clindamycin therapy (Livengood et al). 1990;163:515-20
A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4
Pregnancy outcomes among mothers infected with human immunodeficiency virus and uninfected control subjects (Minkoff et al). 1990;163:1598-604
Sexually transmitted diseases and human immunodeficiency virus infection among women with pelvic inflammatory disease (Hoegsberg et al). 1990;163:1135-9
Shirodkar operation; *see* **Cervical incompetence**
Shock
Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy (Esposito et al). 1990;163:572-4
Shoulder dystocia; *see* **Dystocia**
Sickle cell disease; *see* **Anemia, sickle cell**
Sleep, REM
Fetal eye movement assessed with real-time ultrasonography: Are there rapid and slow eye movements? (Horimoto et al). 1990;163:1480-4
Smoking
Risk factors for preterm premature rupture of fetal membranes: a multicenter case-control study (Harger et al). 1990;163:130-7
Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women (Cassidenti et al). 1990;163:1953-60
Smoking and the antioxidant ascorbic acid: plasma, leukocyte, and cervicovaginal cell concentrations in normal healthy women (Basu et al). 1990;163:1948-52
Smooth muscle; *see* **Muscle, smooth**
Society of Perinatal Obstetricians
Transactions of the tenth annual meeting of the Society of Perinatal Obstetricians. 1990;163:715-999
Sodium
Intracellular sodium levels in pregnant women with hypertension (Goodlin) (Letter); (Sowers and Zemel) (Reply). 1990;163:261-2
Sodium nitroprusside; *see* **Nitroprusside**
South Atlantic Association of Obstetricians and Gynecologists
Retrospection and prospection (McLeod). 1990;163:1000-6 (Pres. address)
Transactions of the fifty-second annual meeting of the South Atlantic Association of Obstetricians and Gynecologists. 1990;163:1000-88
Sperm immobilizing agents
A comparison of sperm antibody assays (Daru and Mathur). 1990;163:1622-9
Sperm motility
How sperm commence movement and their isolation for in vitro fertilization and sex selection (Shettles). 1990;163:270 (Letter)
Sperm-ovum interactions, animal
In vitro effect of RU 486 on sperm-egg interaction in mice (Juneja and Dodson). 1990;163:216-21
Spermatocidal agents
A conceptual thought (Pearse). 1990;163:1098 (Letter)
A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4
Spermatozoa
Antisperm antibodies in women undergoing intrauterine insemination (Goldberg et al). 1990;163:65-8
A comparison of sperm antibody assays (Daru and Mathur). 1990;163:1622-9
Lectins and heparin-binding features of human spermatozoa as analyzed by flow cytometry (Vasquez et al). 1990;163:2006-12
Lymphocyte proliferative response to fertilization antigen in patients with antisperm antibodies (Naz et al). 1990;163:610-3
Spina bifida
The effect of adjusting maternal serum α-fetoprotein levels for maternal weight in pregnancies with fetal open spina bifida (Johnson et al). 1990;163:9-11
Sprague-Dawley rats; *see* **Rats, inbred strains**
Stains and staining
Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients (Towers et al). 1990;163:965-7
Gram stain results from amniocentesis in patients with preterm permature rupture of membranes—comparison of maternal and fetal characteristics (Asrat et al). 1990;163:887-9
Stamey procedure; *see* **Suspensions**
Statistical analysis; *see* **Factor analysis, statistical**
Sterilization, tubal
Necrotizing fasciitis of the total abdominal wall after sterilization by partial salpingectomy (Cederna et al). 1990;163:138-9
Steroids
Neuromodulatory regulation of gonadotropin-releasing hormone pulsatile discharge in women (Jaffe et al). 1990;163:1727-31 (Supplement)
Steroids, sex; *see* **Contraceptives, oral**
Stillbirth; *see* **Fetal death**
Streptococcus agalactiae
Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients (Towers et al). 1990;163:965-7

Cost-effectiveness of intrapartum screening and treatment for maternal group B streptococci colonization (Strickland et al). 1990;163:4-8 (Clin. opinion)
Perinatal mortality in Victoria, Australia: role of group B streptococcus (Fliegner and Garland). 1990;163:1609-11
Postpartum osteomyelitis caused by group B streptococcus (Berkowitz and McCaffrey). 1990;163:1200-1
Risk factors for perinatal group B streptococcal disease after amniotic fluid colonization (Silver et al). 1990;163:19-25

Stress incontinence, urinary; *see* **Urinary incontinence, stress**

Stroke; *see* **Cerebrovascular disorders**

Stroma ovarii, pathology
Specific binding sites for insulin-like growth factor I in the ovarian stroma of women with polycystic ovarian disease and stromal hyperthecosis (Nagamani and Stuart). 1990;163:1992-7

Stromal hyperthecosis; *see* **Stroma ovarii, pathology**

Stuck twins; *see* **Twins**

Subcutaneous hormone implants; *see* **Estrogen replacement therapy**

Substance abuse
Lack of specific placental abnormality associated with cocaine use (Gilbert et al). 1990;163:998-9
The perinatal impact of cocaine, amphetamine, and opiate use detected by universal intrapartum screening (Gillogley et al). 1990;163:1535-42
Prevalence of use of cocaine and other substances in an obstetric population (Matera et al). 1990;163:797-801
Progesterone increases cardiovascular toxicity to cocaine in nonpregnant ewes (Plessinger and Woods). 1990;163:1659-64

Sulfite oxidases
Antenatal diagnosis of molybdenum cofactor deficiency (George et al). 1990;163:1203-4

Supreme court decisions; *see* **Jurisprudence**

Surface-active agents
Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests (Kjos et al). 1990;163:898-903

Surface glycoproteins; *see* **Membrane glycoproteins**

Surfactants; *see* **Surface-active agents**

Surgery, operative
Adhesion formation after ovarian wound repair in New Zealand white rabbits: a comparison of ovarian microsurgical closure with ovarian nonclosure (Wiskind et al). 1990;163:1674-8
Continuous or interrupted fascial closure: a prospective evaluation of No.1 Maxon suture in 402 gynecologic procedures (Orr et al). 1990;163:1485-9
A convenient method to identify prior surgical procedures (Letterie et al). 1990;163:1100 (Letter)
The Maylard incision in gynecologic cancer (Helmkamp and Krebs). 1990;163:1554-7
Surgical staples in cesarean section: a randomized controlled trial (Villeneuve et al). 1990;163:1641-6

Surgical instruments
Fluid cooling of artificial sapphire tips of laser (Loffer) (Letter); (Baggish) (Reply). 1990;163:681
Preventing cervical conization by achieving satisfactory colposcopy with hygroscopic cervical dilators (Stern et al). 1990;163:176-7
Sapphire probes in obstetric laser surgery (Corson) (Letter); (Baggish and Daniell) (Reply). 1990;163:246-7

Surgical menopause, animal; *see* **Menopause, premature, animal**

Surgical staplers
Surgical staples in cesarean section: a randomized controlled trial (Villeneuve et al). 1990;163:1641-6

Surgical techniques and equipment; *see* **Surgery, operative**

Surrogate mothers
Surrogate parenting contracts (Wettstein) (Letter); (La Puma et al) (Reply). 1990;163:679-80

Survival
Mortality rates ignore population segment (Harkavy) (Letter); (Resnick and Ariet) (Reply). 1990;163:269-70
Perinatal survival with expectant management of midtrimester rupture of membranes (Major and Kitzmiller). 1990;163:838-44
Prolongation of pregnancy and survival of second twin (Goodlin) (Letter); (Feichtinger) (Reply). 1990;163:270-1
Survival and outcome of infants weighing <800 grams at birth (Lipper et al). 1990;163:146-50
Survival rates of monoamniotic twins do not decrease after 30 weeks' gestation (Carr et al). 1990;163:719-22

Suspensions
Retropubic long-needle suspension procedures for stress urinary incontinence (Varner). 1990;163:551-7

Sutures
Continuous or interrupted fascial closure: a prospective evaluation of No.1 Maxon suture in 402 gynecologic procedures (Orr et al). 1990;163:1485-9

Sympathomimetics
Role of the endothelium in adrenergic responses of mesenteric artery rings of pregnant rats (Parent et al). 1990;163:229-34

Symphysiotomy
Safe motherhood: Cesarean section or symphysiotomy? (van Roosmalen). 1990;163:1-4 (Clin. opinion)

Syphilis
False-negative syphilis screening: the prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy (Berkowitz et al). 1990;163:975-7
The relationship of cocaine use to syphilis and human immunodeficiency virus infections among inner city parturient women (Minkoff et al). 1990;163:521-6

T

T-ACE questions; *see* **Questionnaires**

T-lymphocytes
Soluble factors produced by isolated first-trimester chorionic villi directly inhibit proliferation of T cells (Silver et al). 1990;163:1914-9

TAG 72; *see* **Antigens, tumor-associated, carbohydrate**

Telemetry
Blood pressure measured telemetrically from home throughout pregnancy (Mooney et al). 1990;163:30-6

Teratogens; *see also* **Carcinogens**
Correlations between antepartum maternal metabolism and newborn behavior (Rizzo et al). 1990;163:1458-64
Embryofetal effects of pentamidine isethionate administered to pregnant Sprague-Dawley rats (Harstad et al). 1990;163:912-6
Perception of teratogenic risk by pregnant women exposed to diagnostic radiation during pregnancy (Mastroiacovo et al) (Letter); (Koren) (Reply). 1990;163:695-6
Pregnancy in a patient with treated Wilson's disease: a case report (Dupont et al). 1990;163:1527-8

Teratoma
Survival in a fetus with sacrococcygeal teratoma and hydrops (Nakayama). 1990;163:682 (Letter)
Terbutaline
Diabetic ketoacidosis and insulin resistance with subcutaneous terbutaline infusion: a case report (Tibaldi et al). 1990;163:509-10
Effect of tocolytic agents on fetal umbilical velocimetry (Wright et al). 1990;163:748-50
A prospective randomized comparison of oral terbutaline and magnesium oxide for the maintenance of tocolysis (Ridgway et al). 1990;163:879-82
Testosterone
Autogenic masculinization (Punch and Ansbacher). 1990;163:114-6
Suppression of serum insulin level by diazoxide does not alter serum testosterone of sex hormone–binding globulin levels in healthy, nonobese women (Nestler et al). 1990;163:1243-6
Thorax
Prenatal diagnosis of a chest wall mass with ultrasonography and Doppler velocimetry: a case report (Smith et al). 1990;163:567-9
Thrombin
Clinical aspects of the relationship between oral contraceptives and abnormalities of the hemostatic system: relation to the development of cardiovascular disease (Kelleher). 1990;163:392-5 (Supplement)
Thrombocytopenia
Continuing controversy in alloimmune thrombocytopenia: fetal hyperimmunoglobulinemia fails to prevent thrombocytopenia (Nicolini et al). 1990;163:1144-6
Cordocentesis and immune thrombocytopenia—continued (Weiner). 1990;163:1371-2 (Letter)
The natural history of thrombocytopenia associated with preeclampsia (Katz et al). 1990;163:1142-3
Responsibility expands to extended family members (Saving et al). 1990;163:1369-70 (Letter)
Thrombocytopenia purpura; *see* **Purpura, thrombocytopenic**
Thromboembolectomy; *see* **Thrombosis**
Thrombosis
Disorders of the hemostatic system and the risk of the development of thrombotic and cardiovascular diseases: limitations of laboratory diagnosis (Kluft). 1990;163:305-12 (Supplement)
Interrelations between carbohydrates, lipids, and the hemostatic system in relation to the risk of thrombotic and cardiovascular disease (Juhan-Vague and Vague). 1990;163:313-5 (Supplement)
Oral contraception in disease states (Breckwoldt et al). 1990;163:2213-6 (Symposium)
Successful treatment of postpartum shock caused by amniotic fluid embolism with cardiopulmonary bypass and pulmonary artery thromboembolectomy (Esposito et al). 1990;163:572-4
Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level (Jauniaux et al). 1990;163:1558-60
Thromboxane
Placental thromboxane and prostacyclin production in an ovine diabetic model (Dickinson et al). 1990;163:1831-5
Thromboxane A_2, analog
Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy (Skajaa et al). 1990;163:1323-33
Thromboxane synthetase, antagonists and inhibitors
Fetal and maternal response to intravenous infusion of a thromboxane synthetase inhibitor (Cardin et al). 1990;163:1345-9
Thyrotropin releasing hormone
Dose response of thyrotropin-releasing hormone on pulmonary maturation in corticosteroid-treated preterm rabbits (Tabor et al). 1990;163:669-76
Thyroxine
Screening infertile women for subclinical hypothyroidism (Strickland et al) (Letter); (Wilansky and Greisman) (Reply). 1990;163:262-3
Tissue culture
Epithelial cells from normal human endometrium express a tumor-associated glycoprotein (TAG-72) epitope in vitro (Osteen et al). 1990;163:479-84
Tissue donors
Anencephalics as organ donors (Van Assche). 1990;163:599-600
Tissue-type plasminogen activator; *see* **Plasminogen activator, tissue-type**
Tocolysis
Adjusting the loading dose of magnesium sulfate for tocolysis (Wright et al). 1990;163:889-92
Awaiting cervical change for the diagnosis of preterm labor does not compromise the efficacy of ritodrine tocolysis (Utter et al). 1990;163:882-6
A comparison of tocolysis with nifedipine or ritodrine: analysis of efficacy and maternal, fetal, and neonatal outcome (Ferguson et al). 1990;163:105-11
Effects of ritodrine tocolysis on cardiac isozymes and electrocardiography (Hendricks and Katz) (Letter); (Hadi and Albazzaz) (Reply). 1990;163:699-701
A prospective randomized comparison of oral terbutaline and magnesium oxide for the maintenance of tocolysis (Ridgway et al). 1990;163:879-82
Uterine hyperstimulation after low-dose prostaglandin E_2 therapy: tocolytic treatment in 181 cases (Egarter et al). 1990;163:794-6
Tocolytic agents
A comparison of tocolysis with nifedipine or ritodrine: analysis of efficacy and maternal, fetal, and neonatal outcome (Ferguson et al). 1990;163:105-11
Effect of tocolytic agents on fetal umbilical velocimetry (Wright et al). 1990;163:748-50
Effects of ritodrine tocolysis on cardiac isozymes and electrocardiography (Hendricks and Katz) (Letter); (Hadi and Albazzaz) (Reply). 1990;163:699-701
Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist (Wilson et al). 1990;163:1875-82
A new tocolytic agent: development of an oxytocin antagonist for inhibiting uterine contractions (Wilson et al). 1990;163:195-202
Tomography, x-ray computed
Prenatal diagnosis of fetal intracranial calcifications (Koga et al). 1990;163:1543-5
Toxicology
Prevalence of use of cocaine and other substances in an obstetric population (Matera et al). 1990;163:797-801
Toxoplasmosis, congenital
Detection of congenital toxoplasmosis by chorionic villus

sampling and early amniocentesis (Foulon et al). 1990;163:1511-3

Transabdominal ultrasonography; *see* **Ultrasonic diagnosis**

Transactions

Transactions of the fifty-second annual meeting of the South Atlantic Association of Obstetricians and Gynecologists. 1990;163:1000-88

Transactions of the tenth annual meeting of the Society of Perinatal Obstetricians. 1990;163:715-999

Transcervical chorionic villus sampling; *see* **Chorionic villi sampling**

Transcortin

Protein binding of active ingredients and comparison of serum ethinyl estradiol sex hormone–binding globulin, corticosteroid-binding globulin, and cortisol levels in women using a combination of gestodene/ethinyl estradiol (Femovan) or a combination of desogestrel/ethinyl estradiol (Marvelon) and single-dose ethinyl estradiol bioequivalence from both oral contraceptives (Hümpel et al). 1990;163:329-33 (Supplement)

Transforming growth factors

Interleukin-1β, human leukocyte antigen DR1, and transforming growth factor-β expression in endometrium, placenta, and placental membranes (Kauma et al). 1990;163:1430-7

Transplacental exposure; *see* **Maternal-fetal exchange**

Transplantation

Anencephalics as organ donors (Van Assche). 1990;163:599-600

Transvaginal ultrasonography; *see* **Ultrasonic diagnosis**

Trial of labor

Reducing cesarean sections at a teaching hospital (Sanchez-Ramos et al). 1990;163:1081-8

Trichomonas vaginitis

Bacterial vaginosis and trichomoniasis vaginitis are risk factors for cuff cellulitis after abdominal hysterectomy (Soper et al). 1990;163:1016-23

A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4

Triplets

Credit neonatal or obstetric care? (Weissman and Jakobi) (Letter); (Milligan) (Reply). 1990;163:1716

Trisomy

The association of single umbilical artery with cytogenetically abnormal pregnancies (Saller et al). 1990;163:922-5

Growth of linear parameters in trisomy 18 fetuses (Droste et al). 1990;163:158-61

Maternal serum Down syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin (Macri et al). 1990;163:1248-53

Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femur length ratio (Marquette et al). 1990;163:1604-5

Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21 (Ginsberg et al). 1990;163:1186-90

Trophoblast

HBcAg identification in the placental cytotypes of symptom-free HBsAg-carrier mothers: a study with the immunoperoxidase method (Lucifora et al). 1990;163:235-9

Inhibition of aromatase activity by 8-bromo-cyclic adenosine monophosphate in cultured first-trimester human trophoblast (Rodway et al). 1990;163:1546-51

Soluble factors produced by isolated first-trimester chorionic villi directly inhibit proliferation of T cells (Silver et al). 1990;163:1914-9

Trophoblastic tumor

Prediction of residual trophoblastic tissue in first-trimester abortions: the role of transvaginal Doppler ultrasonography (Reuwen). 1990;163:2024-5 (Letter)

Tuberculosis, female genital

An abdominal pregnancy 10 years after treatment for pelvic tuberculosis (Durukan et al). 1990;163:594-5

Tubocurarine

Differential sensitivities of fetal muscle groups to *d*-tubocurarine (Szeto and Hinman). 1990;163:202-9

Tumor antigens; *see* **Antigens, neoplasm**

Tumor-associated glycoproteins; *see* **Membrane glycoproteins**

Tumor necrosis factor

Proliferative and antiproliferative effects of interferon-γ and tumor necrosis factor-α on cell lines derived from cervical and ovarian malignancies (Mutch et al). 1990;163:1920-4

Serum levels of interleukin-2 and tumor necrosis factor α in women with recurrent abortion (Mallmann et al). 1990;163:1367 (Letter)

Tumorigenicity tests; *see* **Carcinogens**

Turner's syndrome

Correlation of the testicular determinant factor sequence zinc finger Y with varying gonadal phenotypes in a series of 13 subjects with gonadal dysgenesis because of Y aneuploidy (Tho et al). 1990;163:1968-75

Twin-to-twin transfusion; *see* **Fetofetal transfusion**

Twin transfusion; *see* **Fetofetal transfusion**

Twins

Fetal Doppler velocimetry in twins (Degani) (Letter); (Divon) (Reply). 1990;163:1100-1

Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in twin gestations with preterm labor (Romero et al). 1990;163:757-61

Prognosis for twins with birth weight <1500 gm: the impact of cesarean section in relation to fetal presentation (Rydhström). 1990;163:528-33

Prolongation of pregnancy and survival of second twin (Goodlin) (Letter); (Feichtinger) (Reply). 1990;163:270-1

The stuck twin phenomenon: Ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses (Mahony et al). 1990;163:1513-22

Twin-twin transfusion syndrome (Urig et al). 1990;163:1522-6

Twins, monozygotic

Perinatal outcome of forty-nine pregnancies complicated by acardiac twinning (Moore et al). 1990;163:907-12

Survival rates of monoamniotic twins do not decrease after 30 weeks' gestation (Carr et al). 1990;163:719-22

U

Ulcer, vulvar; *see* **Vulvar diseases**

Ultrasonic diagnosis

Accuracy of apical four-chamber view as screen for congenital heart disease (Stephens). 1990;163:249-50 (Letter)

Appropriate screening tests (Nagey) (Letter); (Chervenak et al) (Reply). 1990;163:688-9

Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry (Ikeda and Mori). 1990;163:494-8

Blood flow velocity and pulsatile diameter changes in the

Ultrasonic diagnosis—cont'd
fetal descending aorta: a longitudinal study (Stale et al). 1990;163:26-9
Color flow Doppler—a useful instrument in the diagnosis of vasa previa (Harding et al). 1990;163:1566-8
The diagnostic values of concurrent nonstress testing, amniotic fluid measurement, and Doppler velocimetry in screening a general high-risk population (Devoe et al). 1990;163:1040-8
Direct ultrasonographic measurement of fetal lung length in normal pregnancies and pregnancies complicated by prolonged rupture of membranes (Roberts and Mitchell). 1990;163:1560-6
Does the umbilical artery systolic/diastolic ratio reflect flow or acidosis? (Copel et al). 1990;163:751-6
Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development (van Eyck et al). 1990;163:558-66
The effect of nifedipine therapy on fetal and placental Doppler waveforms in preeclampsia remote from term (Moretti et al). 1990;163:1844-8
Effects of intravascular fetal blood transfusion on fetal intracardiac Doppler velocity waveforms (Rizzo et al). 1990;163:1231-8
Endometrial assessment by vaginal ultrasonography before endometrial sampling in patients with postmenopausal bleeding (Goldstein et al). 1990;163:119-23
Ethics and routine ultrasonography in pregnancy (Ewigman et al) (Letter); (Chervenak et al) (Reply). 1990;163:256-8
Fetal Doppler velocimetry in twins (Degani) (Letter); (Divon) (Reply). 1990;163:1100-1
Fetal eye movement assessed with real-time ultrasonography: Are there rapid and slow eye movements? (Horimoto et al). 1990;163:1480-4
Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive value for pregnancy-induced hypertension (Hume et al). 1990;163:826-9
Human fetal foramen ovale flow velocity waveforms relative to behavioral states in normal term pregnancy (van Eyck et al). 1990;163:1239-42
Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1990;163:1313-20
In utero detection of cardiac failure from an aneurysm of the vein of Galen (Jeanty et al). 1990;163:50-1
Infants of diabetic mothers with accelerated fetal growth by ultrasonography: Are they all alike? (Keller et al). 1990;163:893-7
Observations on the cause of oligohydramnios in prolonged pregnancy (Trimmer et al). 1990;163:1900-3
Prediction of residual trophoblastic tissue in first-trimester abortions: the role of transvaginal Doppler ultrasonography (Reuwen). 1990;163:2024-5 (Letter)
Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length (Andersen et al). 1990;163:859-67
Prenatal diagnosis of a chest wall mass with ultrasonography and Doppler velocimetry: a case report (Smith et al). 1990;163:567-9
Relationship between the ovine fetal umbilical artery blood flow waveform and umbilical vascular resistance (Irion and Clark). 1990;163:222-9
Reliability of ultrasonographic formulary in the prediction of fetal weight and survival of very-low-birth-weight infants (Mills et al). 1990;163:1568-74
Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femur length ratio (Marquette et al). 1990;163:1604-5
Second-trimester ultrasonography: determinants of visualization of fetal anatomic structures (Catanzarite and Quirk). 1990;163:1191-5
Spontaneous resolution of a nuchal fetal cystic hygroma diagnosed early in the second trimester of pregnancy (Meizner et al) (Letter); (Bronshstein and Blumenfeld) (Reply). 1990;163:267-9
Superiority of the four-quadrant sum over the single-deepest-pocket technique in ultrasonographic identification of abnormal amniotic fluid volumes (Moore). 1990;163:762-7
Transabdominal and transvaginal ultrasonography in the diagnosis of ectopic pregnancy: a comparative study (Kivikoski et al). 1990;163:123-8
Transvaginal ultrasonographic detection of congenital anomalies in the first trimester (Cullen et al). 1990;163:466-76
Ultrasonographic assessment of intestinal damage in fetuses with gastroschisis: Is it of clinical value? (Lenke et al). 1990;163:995-8
Ultrasonographic assessment of placental abnormalities (Jauniaux and Campbell). 1990;163:1650-8
Ultrasonographic dating of very early pregnancy (Cadkin and McAlpin-Cadkin). 1990;163:1103 (Letter)
Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21 (Ginsberg et al). 1990;163:1186-90
Ultrasonographic diagnosis of a large placental intervillous thrombosis associated with elevated maternal serum α-fetoprotein level (Jauniaux et al). 1990;163:1558-60
Ultrasonographic evaluation of uteroplacental blood flow patterns of abnormally located and adherent placentas (Guy et al). 1990;163:723-7
Ultrasonographic fetal weight estimation by an integrated computer-assisted system: Can each laboratory improve its accuracy? (Thompson et al). 1990;163:986-95
Ultrasonographic guidance for central venous access (Greenspoon) (Letter); (Lee) (Reply). 1990;163:252
Ultrasonography's role in detection of intrauterine synechiae (Stephens) (Letter); (Valle and Sciarra) (Reply). 1990;163:1106-7
Vaginal probe ultrasonography and ectopic pregnancy (Goldstein) (Letter); (Stiller) (Reply). 1990;163:1099-100
Variation and correlation in human fetal umbilical Doppler velocities with fetal breathing: evidence of the cardiac-placental connection (Indik and Reed). 1990;163:1792-6

Umbilical arteries
The association of single umbilical artery with cytogenetically abnormal pregnancies (Saller et al). 1990;163:922-5
Comparison of umbilical artery pH and 5-minute Apgar score in the low-birth-weight and very-low-birth-weight infant (Stark et al). 1990;163:818-23
Effect of tocolytic agents on fetal umbilical velocimetry (Wright et al). 1990;163:748-50
Fetal umbilical artery Doppler response to graded maternal aerobic exercise and subsequent maternal mean arterial blood pressure: predictive value for pregnancy-induced hypertension (Hume et al). 1990;163:826-9
Sources of variability in umbilical artery systolic/diastolic ratios: implications of Poiseuille equation (Wright and Ridgway). 1990;163:1788-91
Variation and correlation in human fetal umbilical Doppler

velocities with fetal breathing: evidence of the cardiac-placental connection (Indik and Reed). 1990;163:1792-6

Umbilical arteries, animal

Does the umbilical artery systolic/diastolic ratio reflect flow or acidosis? (Copel et al). 1990;163:751-6

Hypoxic acidemia, hyperviscosity, and maternal hypertension do not affect the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1990;163:1313-20

Relationship between the ovine fetal umbilical artery blood flow waveform and umbilical vascular resistance (Irion and Clark). 1990;163:222-9

Umbilical cord

A comparison of the effect of epidural, general, and no anesthesia on funic acid-base values by stage of labor and type of delivery (Shyken et al). 1990;163:802-7

Effects of magnesium and isradipine on contractile activation induced by the thromboxane A_2 analog U46619 in human uteroplacental arteries in term pregnancy (Skajaa et al). 1990;163:1323-33

Sources of variability in umbilical artery systolic/diastolic ratios: implications of Poiseuille equation (Wright and Ridgway). 1990;163:1788-91

Umbilical veins

Acute fetal hemodynamic alterations after intrauterine transfusion for treatment of severe red cell alloimmunization (Moise et al). 1990;163:776-84

Umbilical velocimetry; *see* **Flowmeters**

Universal screening; *see* **Mass screening**

Ureaplasma

Cervicovaginal microflora and pregnancy outcome: results of a double-blind, placebo-controlled trial of erythromycin treatment (McGregor et al). 1990;163:1580-91

Urinary calcium; *see* **Calcium**

Urinary incontinence

The effect of large uterine fibroids on urinary bladder function symptoms (Langer et al). 1990;163:1139-41

Urinary incontinence, stress

The effect of large uterine fibroids on urinary bladder function symptoms (Langer et al). 1990;163:1139-41

Retropubic long-needle suspension procedures for stress urinary incontinence (Varner). 1990;163:551-7

Suprapubic or vaginal procedure (Cabrera et al) (Letter); (Bergman et al) (Reply). 1990;163:2025-6

Uterine catheterization

Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle (Figueroa et al). 1990;163:648-54

Uterine contraction

The effect of amnioinfusion on uterine pressure and activity: a preliminary report (Posner et al). 1990;163:813-8

Gestational changes in mechanical properties of skinned muscle tissues of human myometrium (Izumi et al). 1990;163:638-47

A new tocolytic agent: development of an oxytocin antagonist for inhibiting uterine contractions (Wilson et al). 1990;163:195-202

Studies of the spontaneous motility and the effect of histamine on isolated myometrial strips of the nonpregnant human uterus: the influence of various uterine abnormalities (Martinez-Mir et al). 1990;163:189-95

Uterine contraction, animal

Inhibition of spontaneous uterine contractions during the last trimester in pregnant baboons by an oxytocin antagonist (Wilson et al). 1990;163:1875-82

A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios (Harding et al). 1990;163:1904-13

Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy (Isla and Dyer). 1990;163:1337-44

Uterine hemorrhage

Oral contraceptives in risk groups: Exclusion or monitoring? (Guillebaud). 1990;163:443-6 (Supplement)

Uterine hyperstimulation; *see* **Labor complications**

Uterine neoplasms

The effect of large uterine fibroids on urinary bladder function symptoms (Langer et al). 1990;163:1139-41

Efficacy and safety considerations in women with uterine leiomyomas treated with gonadotropin-releasing hormone agonists: the estrogen threshold hypothesis (Friedman et al). 1990;163:1114-9 (Clin. opinion)

Experience with the EndoPap device for the cytologic detection of uterine cancer and its precursors: a comparison of the EndoPap with fractional curettage or hysterectomy (LaPolla et al). 1990;163:1055-60

Preoperative evaluation of serum CA 125, TAG 72, and CA 15-3 in patients with endometrial carcinoma (Soper et al). 1990;163:1204-9

Uterine prolapse

A vesicovaginal fistula and intravesical foreign body: a rare case of the neglected pessary (Goldstein et al). 1990;163:589-91

Uterine rupture

Catastrophic complications of previous cesarean section (Chazotte and Cohen). 1990;163:738-42

Intrapartum uterine rupture and inflammatory bowel disease (Peterson and Braak). 1990;163:1247-8

Rupture of unscarred uterus in primigravid woman in association with cocaine abuse (Gonsoulin et al). 1990;163:526-7

Uteroplacental blood flow; *see* **Placenta**

Uterus

The effect of amnioinfusion on uterine pressure and activity: a preliminary report (Posner et al). 1990;163:813-8

Experience with the EndoPap device for the cytologic detection of uterine cancer and its precursors: a comparison of the EndoPap with fractional curettage or hysterectomy (LaPolla et al). 1990;163:1055-60

Laparoscopic ventrosuspensions (Yoong). 1990;163:1151-3

Modified laparoscopy for monitoring transcervical surgery (Lin and Iwata). 1990;163:243-4 (Letter)

Studies of the spontaneous motility and the effect of histamine on isolated myometrial strips of the nonpregnant human uterus: the influence of various uterine abnormalities (Martinez-Mir et al). 1990;163:189-95

Uterine artery estrogen receptors in the nonpregnant and pregnant guinea pig (Leiberman et al). 1990;163:1685-8

Uterus, animal

Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy (Isla and Dyer). 1990;163:1337-44

V

Vacuum extraction

Risk factors for third-degree and fourth-degree perineal lacerations in forceps and vacuum deliveries (Combs et al). 1990;163:100-4

Vagina
Endometrial assessment by vaginal ultrasonography before endometrial sampling in patients with postmenopausal bleeding (Goldstein et al). 1990;163:119-23
Vaginal diseases
Bacterial vaginosis: diagnostic and pathogenetic findings during topical clindamycin therapy (Livengood et al). 1990;163:515-20
A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4
Vaginitis
Bacterial vaginosis: diagnostic and pathogenetic findings during topical clindamycin therapy (Livengood et al). 1990;163:515-20
Bacterial vaginosis and trichomoniasis vaginitis are risk factors for cuff cellulitis after abdominal hysterectomy (Soper et al). 1990;163:1016-23
A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis (Barbone et al). 1990;163:510-4
Valve prosthesis; *see* **Heart valve prosthesis**
Vanable decelerations; *see* **Heart rate, fetal**
Vancomycin, adverse effects
Toxicity of vancomycin given during pregnancy (Gouyon and Petion) (Letter); (Reyes and Ostrea) (Reply). 1990;163:1375-6
Varicella-zoster virus
Extensive vulvar and vaginal varicella necessitating abdominal delivery (Sites et al). 1990;163:1630-1
Vasa previa; *see* **Placenta praevia**
Vascular access ports; *see* **Catheters, indwelling**
Vascular resistance
Response of placental vasculature to high glucose levels in the isolated human placental cotyledon (Roth et al). 1990;163:1828-30
Vascular resistance, animal
Maternal and uteroplacental hemodynamic effects of chronic captopril in the hypertensive, term-pregnant rat (Mabie et al). 1990;163:1861-7
Relationship between the ovine fetal umbilical artery blood flow waveform and umbilical vascular resistance (Irion and Clark). 1990;163:222-9
Vasopressin; *see* **Argipressin**
Oxytocin and vasopressin binding sites in human and bovine ovaries (Fuchs et al). 1990;163:1961-7
Vein of Galen; *see* **Cerebral veins**
Velocimetry; *see* **Flowmeters**
Venous access; *see* **Catheters, indwelling**
Verapamil
Vasodilatory effects of nifedipine, methoxyverapamil, and sodium nitroprusside on contractile responses of the ewe uterine artery at term pregnancy (Isla and Dyer). 1990;163:1337-44
Vesicovaginal fistula
A vesicovaginal fistula and intravesical foreign body: a rare case of the neglected pessary (Goldstein et al). 1990;163:589-91
Viability of fetus; *see* **Fetal viability**
Vibroacoustic stimulation; *see* **Acoustic stimulation**
Vision disorders
Neuroophthalmologic effects of intravenous magnesium sulfate (Digre et al). 1990;163:1848-52
Vitamin C; *see* **Ascorbic acid**
Vitamin E
Vitamin E levels in premenstrual syndrome (Chuong et al). 1990;163:1591-5
von Hippel-Lindau disease; *see* **Hippel-Lindau disease**
von Willebrand factor
Hemostasis profile in women taking low-dose oral contraceptives (David et al). 1990;163:420-3 (Supplement)
Vulvar diseases
Autogenic masculinization (Punch and Ansbacher). 1990;163:114-6
Vulvar ulcer of unknown etiology in a human immunodeficiency virus–infected woman: response to treatment with zidovudine (Covino and McCormack). 1990;163:116-8
Vulvar neoplasms
Invasive carcinoma of the vulva (Cavanagh et al). 1990;163:1007-15

W

Webster vs Reproductive Health Services; *see* **Jurisprudence**
Wilson's disease; *see* **Hepatolenticular degeneration**

X

X chromosome
Cytogenetic uncertainties surrounding the fragile X in Martin-Bell syndrome (Sindwani and Verma). 1990;163:1713-4 (Letter)

Z

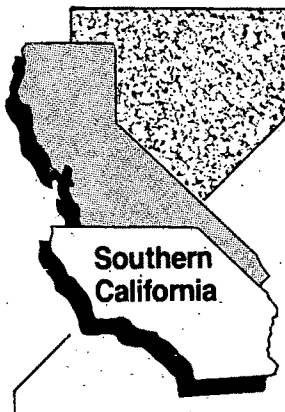
Zidovudine
Transplacental transfer of zidovudine in the near-term pregnant baboon (Hankins et al). 1990;163:728-32
Vulvar ulcer of unknown etiology in a human immunodeficiency virus–infected woman: response to treatment with zidovudine (Covino and McCormack). 1990;163:116-8
Zinc
Zinc, preeclampsia, and γ-aminobutyric acid (Brophy). 1990;163:242-3 (Letter)

Outstanding Obstetrics-Gynecology practice opportunity!

Located on Ohio's North coast with the best of four seasons in a city with excellent schools and cultural attractions.

600+ bed tertiary care medical center. Comprehensive services provided in a growing OB/GYN department. Excellent clinical and diagnostic facilities. Very competitive financial package available for qualified BE/BC physician.

Interested individuals may send CV in confidence to:
Ellen F. Yunker
Manager, Physician Recruitment,
St. Vincent Medical Center
2213 Cherry Street
Toledo, OH 43608-2691
or call (419) 321-4462



Join The Partnership of Choice

Southern California Permanente Medical Group (SCPMG) is recognized as the nation's largest and most respected physician-managed, multi-specialty medical group.

Our continued growth has created new opportunities for:

OBSTETRICIANS & GYNECOLOGISTS

You'll have a choice of professional environments ranging from a 250-bed community hospital to a major teaching facility in a number of prime Southern California locations. Our active practices require proficiency in modern ultrasound and surgical techniques.

CHIEF OF SERVICE

An excellent opportunity for an experienced OB/GYN practitioner to lead an established and growing practice at our Panorama City Medical Center. You'll be responsible for supervising 20 OB/GYN Physicians, a Perinatologist, 8 Certified Nurse Midwives and 4 Nurse Practitioners. This team of professionals will handle approximately 3000 deliveries per year.

SCPMG provides the freedom, technology and resources to focus on quality patient care and the collaborative support of knowledgeable colleagues. **We also provide a highly competitive salary, paid malpractice and predictable hours.**

KAISER PERMANENTE
Southern California Permanente Medical Group

Partners Practicing Good Medicine

Call or send your CV to:
Irwin P. Goldstein, M.D., Associate Medical Director
SCPMG, Dept. 006
Walnut Center, Pasadena, CA 91188-8013
Or Call: 1-800-541-7946

RESIDENCY IN OBSTETRICS/ GYNECOLOGY

The Department of Obstetrics and Gynecology at Akron City Hospital has an ACGME approved position available at the PGY-2 level beginning immediately. Applicants must have received one year of Obstetrics/Gynecology training in a ACGME approved training program.

Send letter of application and curriculum to:

John Karlen, M.D.
Chairman and Residency Director
Akron City Hospital
525 East Market Street
Akron, Ohio 44309

Akron City Hospital is an equal opportunity affirmative action employer.

OBSTETRICIAN/ GYNECOLOGIST

Large, independent multi-specialty group in Minneapolis-St. Paul metropolitan area seeks additional BC/BE obstetrician/gynecologists to join challenging, established practice spanning the full range of obstetrics and gynecology. Competitive salary and benefit package. Send inquiries and curriculum vitaes to:

Nancy Borgstrom
Administration
Aspen Medical Group
1020 Bandana Boulevard West
St. Paul, MN 55108
(612) 641-7185

Equal Opportunity Employer

Finger Lakes Region Ithaca, New York

Unique opportunity for patient oriented physician in comprehensive OB-GYN service, building on established patient base of 6,000 patients. To include out-patient gynecology, obstetrics, surgery, infertility, and abortions. First call protection from nurse-midwives. Progressive university community (home of Cornell University) offers cultural attractions of a city with the benefits of small town living. Competitive salary, excellent benefits including malpractice. Summer/Fall 1991.

Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.

Planned Parenthood®
of Tompkins County

PROFESSOR AND DIRECTOR DIVISION OF GYNECOLOGIC ONCOLOGY

A full time faculty position is available for Professor, and Director of the Division of Gynecological Oncology 1 April 1991. Excellent opportunities exist for teaching and research. A new University Hospital opened in November, 1989. The division has an active clinical service in Parkland Memorial Hospital, and is involved in multiple GOG protocols. There is an approved 3-year fellowship program and the first year consists of laboratory research.

Salary and faculty appointment are commensurate with curriculum vitae and experience. Board certification in gynecological oncology required. Direct all inquiries to: David Hemsell, M.D., Chair, Search Committee, Department Obstetrics & Gynecology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235-9032. An affirmative action/equal opportunity employer.

FULL-TIME FACULTY
OB/GYN GENERALIST

The Department of Obstetrics and Gynecology of the Bowman Gray School of Medicine of Wake Forest University is seeking an additional generalist. The position involves primary responsibility in General Gynecology with teaching and supervision of residents and students in a clinical setting and some private practice. There is opportunity for participation in both clinical and basic research.

The Bowman Gray School of Medicine is located in the Piedmont Triad of North Carolina in an area offering an unusual diversity of cultural and recreational amenities.

Competitive salary, fringe benefit package and academic appointment are offered in accordance with experience. Send CV to:

Eberhard Mueller-Heubach, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
Bowman Gray School of Medicine
Wake Forest University
300 South Hawthorne Road
Winston-Salem, NC 27103

The Wake Forest University is an equal opportunity affirmative action employer. Women and minorities are encouraged to apply.

BRIDGEPORT HOSPITAL
Bridgeport, Connecticut
DEPARTMENT OF OB/GYN
is seeking a full time
3rd PERINATOLOGIST

The department performs approximately 3000 deliveries/ year, with an additional 3000 deliveries performed in neighboring Level I hospitals. There is an accreditated residency program in Obstetrics and Gynecology, along with a Level III Intensive Care Nursery. The section provides high risk obstetrical consultation, obstetrical imaging, genetic counseling. There is a close affiliation with the Yale Department of OB/GYN and abundant opportunities for clinical research.

We are looking for a BC/BE individual with an interest in clinical perinatology and a strong background in perinatal genetics to complement our existing services. The salary and benefits offered by the institution are extremely competitive and Fairfield County is an attractive place to live and raise a family.

Interested candidates should send their CV to:

Robert J. Stiller, M.D.
Director, Maternal-Fetal Medicine
Bridgeport Hospital
267 Grant Street
Bridgeport, CT 06610
(203) 384-3544

Relocate to sunny Southern California.

Christian Hospital Medical Center is an acute care facility located in one of the fastest growing areas with young families and industry.

Opportunities available for OB/GYN physicians in our new women's program from prenatal through postpartum starting December 1990. Be a part of our innovative professional team including complete medical and educational services. These positions boast a competitive benefits package and practice conditions.

The Christian Hospital Medical Center is situated in a growing area within easy reach of Riverside and San Diego, thus offering the best of small town hospitality and large city convenience. Loma Linda is with easy commuting distance for high risk clients.

Please feel free to send your confidential resume to:

Administrator
Christian Hospital Medical Center
2224 Medical Center Drive
Ferris, CA 92370
Phone (714) 657-7381
Fax 714 943-3306

OB-GYN

70-Doctor multispecialty group seeking a board certified/eligible Obstetrician-Gynecologist by June 1991 to associate with 4 MDs in an established practice. Well-equipped offices in a modern expanding facility. Big-10 University community of 100,000, superb cultural advantages, ideal for family. Opportunity for medical school affiliation. Excellent salary and benefits with early associateship. Reply with CV:

Ronald H. Deering
101 West University Avenue
Champaign, IL 61820

COLUMBIA HOSPITAL FOR WOMEN ❀ MEDICAL CENTER

PERINATOLOGIST—WASHINGTON, D.C.

Active, private, OB/GYN/Neonatology speciality hospital with over 5,000 deliveries per year is seeking a Perinatologist for our expanding Division of Maternal Fetal Medicine. We have an excellent 35 bed NICU as well as outstanding ancillary support and excellent departments in other OB/GYN subspecialties. Board certified/board eligible candidates may send resumes to:

Lee Styles
Recruitment and Employment Manager
Columbia Hospital for Women
Medical Center
2425 L Street, NW
Washington, D.C. 20037

Equal Opportunity Employer



COPLEY HOSPITAL DELIVERS

If you do, too, perhaps you'd like to join us. You will be joining a medical staff of 38 primary care and specialty physicians at one of the finest small (53 bed) rural hospitals in the country.

Progressive, creative and award winning programs have made Copley an important name in rural health care. Near Stowe, Vermont, our special setting provides natural beauty and a variety of activities for an unusual population mix—both country and cosmopolitan.

Position available immediately for the right Board Certified or Board Eligible OB/GYN. If you think you're the physician we're looking for, send letter of application and curriculum vitae to Reginald Lavoie, Vice President of Planning & Clinical Services, Copley Hospital, RR #3, Box 760, Morrisville, Vermont 05661.

MATERNAL-FETAL MEDICINE
CREIGHTON UNIVERSITY

Perinatologist needed to join two man Division in growing, rejuvenated Department at a major Jesuit university. Excellent institutional support, two teaching hospitals with good variety of challenges and 4000 deliveries annually. Affiliation with Boys Town National Research Hospital. Metropolitan center with pleasant weather variety, comfortable lifestyle, and low crime rate. Rank and role in the Division negotiable and dependent on experience, interests, and goals to be mutually established. Highly competitive salary and benefits.

Contact:
Richard P. Perkins, M.D.
Professor of Obstetrics and Gynecology
Director of Obstetric Programs
601 N. 30th Street
Omaha, NE 68131

BOARD CERTIFIED OB/GYN

Solo Practitioner seeks a BE/BC associate for growing solo practice located in south St. Louis County, Missouri. Near major interstates. Attractive new office and equipment. Excellent growth potential. Salary plus benefits first year, leading to partnership.

Send Curriculum Vitae to:

David E. Dugger, M.D.
11132 South Towne Sq #104
St. Louis, MO 63123

OB/GYN PHYSICIANS:

SPECIALIZE IN...



Currently we offer exceptional solo and group opportunities for board certified/board eligible OB/GYN Physicians affiliated with DeKalb Medical Center. A 500-bed comprehensive care hospital serving a comfortable community in suburban Atlanta. Site of soccer's World Cup in 1992, football's Superbowl in 1994 and the Olympics in 1996, Atlanta also abounds in cultural and educational resources and affords easy access to both mountain and ocean recreation. Please direct your inquiries and CV's to: **Pamela C. Richards, Director of Medical Staff Development, DeKALB MEDICAL CENTER, 2701 North Decatur Road, Decatur, GA 30033, 1-800-562-9721.** An Equal Opportunity Employer.

Dekalb Medical Center

OBSTETRICIAN GYNECOLOGIST

Fish Memorial Hospital, New Smyrna Beach, Florida, located on the Florida East Coast is seeking a full-time, Board Certified/Eligible Obstetrican/Gynecologist to establish a solo practice or partnership with an existing OB/GYN physician. The hospital is a 116 bed JCAHO accredited facility, in the process of renovating its OB unit. Located 20 minutes south of Daytona Beach and 1 and 1/2 hours away from the Walt Disney/Orlando area. An excellent opportunity exists to develop a financially and professionally rewarding practice. A competitive relocation and financial package is available. For more information please contact:

Carl Abdalian, Administrator
Fish Memorial Hospital
401 Palmetto Street
New Smyrna Beach, Florida 32168
904-427-3401

ELKO REGIONAL MEDICAL CENTER

762 - 14th Street Elko, Nevada 89801 (702) 738-3111

OBSTETRICIAN/GYNECOLOGIST

Nevada's oldest multi-specialty clinic seeks BE/BC OB/GYN to join two other OB-GYN's and Nurse Midwife in very busy practice. Lucrative opportunity for full range of specialty practice. All fee for service with excellent collection ratio. Guaranteed salary for six months leading to full partnership. Comprehensive benefit package including malpractice insurance. Elko is a thriving community surrounded by mountains and wilderness areas. Recreation year round. Please send C.V. to:

Cherie Atwood, Administrator
Elko Regional Medical Center
762-14th Street
Elko, Nevada 89801
(702) 738-3111

Life.

It's our life's work.

If your mission in life is to practice the ideals of medicine without administrative demands, we invite your inquiry.

Group Health Plan in St. Louis is a staff model HMO that promotes the ideals of private practice while freeing doctors from the financial baggage of the private practitioner.

Group Health Plan isn't for every physician. Just for those few who want to practice medicine without compromise.

For an interview please talk with the Medical Recruiter at (314) 993-5955. Or write Group Health Plan, 11475 Olde Cabin Road, St. Louis, Mo. 63141. An equal opportunity employer.

GROUP HEALTH PLAN

CHAIR, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

The Allentown Hospital–Lehigh Valley Hospital Center (TAH-LVHC), an 830-bed regional tertiary care teaching hospital is seeking an OB/GYN Chair. The hospital has 33 active OB/GYN staff physicians, including a gynecologic oncologist and reproductive endocrinologist. The current Chair, who will be leaving at the end of this year, is a perinatologist. The hospital is currently recruiting a maternal-fetal subspecialist. TAH-LVHC is a regional Level III Perinatal Center and a clinical campus of Hahnemann University in Philadelphia. Of over 3,300 births in 1989, 42% were in the high-risk category. The department is supported by a 20-bed Level III NICU and has an active OB/GYN clinic which experienced over 8,000 visits in 1989.

NEWLY-CREATED POSITION OF VICE CHAIR/DIRECTOR OF EDUCATION

In response to the continuing growth of the community and the expansion of the OB/GYN Department, TAH-LVHC is seeking its first full-time OB/GYN Vice Chair/Director of Education. The hospital has a fully-approved free-standing Residency Program with eight American-trained residents. A total of 70 residents and 400 medical students are trained at the hospital each year.

The hospital is located in Allentown, a cosmopolitan city one hour north of Philadelphia and one and one-half hours west of New York City. Interested board-certified candidates may send a curriculum vitae, in confidence, to **Headley S. White, Jr., M.D., Sr. V.P., Medical and Academic Affairs, c/o HealthSearch, 50 College Drive, Suite 323, Allentown, PA 18104. (215)778-7993.**

PERINATOLOGIST

Outstanding practice opportunity for a BC/BE perinatologist to join an established practice at Iowa's largest private teaching medical center. The hospital, a state designated Level III Perinatal Center, averages over 2,000 deliveries annually and has an active maternal and neonatal transport system. The medical center's Level III Neonatal Intensive Care Unit is staffed by four board certified neonatologists. Complete laboratory facilities are available including cytogenetic analysis of both amniotic fluid and trophoblastic tissue. The Antepartum Testing Unit is equipped with computer-based fetal monitors and a color flow Doppler ultrasound unit. Excellent financial and lifestyle opportunities are available to the qualified candidate. Please call 515/283-5911 or send CV to Mariann Clark, Medical Search Consultant, 1200 Pleasant Street, Des Moines, IA 50309.

MATERNAL-FETAL MEDICINE SPECIALIST

Join existing Maternal-Fetal Medicine Specialist and practice at Methodist Hospital of Indiana. Candidates must be fellowship trained in Maternal-Fetal Medicine or Perinatology.

Indianapolis hospital has over 1,100 beds, a Level III nursery, and a Pediatric ICU. This is an extremely busy, existing practice which also offers the opportunity for academics/research.

Compensation includes salary from Methodist Hospital plus private practice income. Total package of over $200,000 for the first year. Generous benefits package available. All interviewing and relocation expenses paid. Send CV or call:

Greg E. Flowers
JACKSON AND COKER, INC.
115 Perimeter Center Place
Suite 380 AJOGO
Atlanta, GA 30346
Tel. 1-800-544-1987



Loyola University Chicago Faculty Positions

Maternal Fetal Medicine

Board Certified/Board eligible physician to join 5 person academic group perinatal practice as a full time faculty member in the Department of Obstetrics and Gynecology.

OB/GYN Generalist

Outstanding opportunity for OB/GYN Board eligible/Board Certified physician who is interested in a full time academic position.

Contact:

John H. Isaacs, M.D., Chairman
Obstetrics and Gynecology Recruitment Committee
Loyola University Chicago
2160 South First Avenue
Maywood, Illinois 60153
(708) 216-3383

LAKE MICHIGAN AREA

OB/GYN to join another OB/GYN in multispecialty group or choose the option to go solo with coverage. There are only 5 OB/GYN's covering a 215,000 service area. Practice is located on Lake Michigan and is only 90 minutes from Chicago in Southwestern Michigan.

330-bed hospital had 1,300 deliveries last year. Facilities include 4 LDR's, a 24-hour, in-house Anesthesia (Epidural Service), and a Level II nursery. Send CV or call:

Ken Sammut
JACKSON AND
COKER, INC.
115 Perimeter Center Place
Suite 330 AJOGO
Atlanta, GA 30346
1-800-544-1987



OB/GYN

Tertiary affiliated, community teaching hospital, in Western Suburb of Chicago, with OB/GYN residency training program, seeks aggressive young BE/BC OB/GYNs to establish in private practice, in our soon to be developed primary care center. Come join us in entering a market where the needs of Women's Healthcare continue to go unfulfilled. If teaching is of interest, our program is for you, offering the opportunity to assist in meeting the training requirements of our residents in your area of expertise. We offer attractive financial support to assist you in the quick and successful development of your private practice. Send CV in confidence to:

P.O. Box 3514
Oak Brook, IL 60522-3514

Ambulatory Gynecologist

Marshfield Clinic's Department of OB/GYN is expanding and is seeking a board-certified obstetrician/gynecologist for a new Ambulatory Gynecology position. Primary responsibilities include walk-in ambulatory patients, performance of diagnostic office procedures, directing the departments' physician extender clinic, and initial infertility evaluations. No OB or GYN call; no obstetrical care. Marshfield Clinic is a 350 physician multi-specialty private group located in a thriving family-oriented community in central Wisconsin. Please send curriculum vitae to:

George L. Hill, M.D.
Chairman OB/GYN Department
1000 North Oak Avenue
Marshfield, WI 54449
or call collect
(715) 387-5161

Marshfield Clinic

FACULTY POSITION MATERNAL-FETAL MEDICINE

The Department of Obstetrics and Gynecology, Eastern Virginia Medical School, is expanding its Division of Maternal-Fetal Medicine. A full time faculty position at the Assistant or Associate Professor level is available for a candidate who is Board eligible/certified in this subspecialty. The job description calls for at least 50% of the time reserved for clinical and/or basic research. The Division, with its Board approved fellowship, serves a population of over one million as the only perinatal center in the Eastern Virginia region. Call or send CV to:

Donald L. Levy, M.D.
Professor and Director
Division of Maternal-Fetal Medicine
Eastern Virginia Medical School
825 Fairfax Avenue
Norfolk, Virginia 23507
(804) 628-7300

OB/GYN

Well established multi-specialty prepaid group practice seeks BC/BE OB/GYN physicians for two of our SUBURBAN BOSTON health centers. Reasonable work and coverage schedules. Attractive well-equipped facilities located within reasonable distance of ski resorts, beaches, and academic and cultural centers. Excellent compensation and fringe benefits package including full malpractice coverage. **Send C.V. to Director of Professional Human Resources, Medical East Community Health Plan, Ste. 200, 99 Darby Street, Hingham, MA 02043-4228; or call collect 617-740-6000.**

Medical East®
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

OBSTETRICIAN GYNECOLOGIST

Western Massachusetts staff model Health Maintenance Organization is seeking two full-time, Board Certified/Board Eligible Obstetrician/Gynecologists to join our expert staff of five physicians and five midwives.

Our multi-specialty group practice employs 70 full-time physicians and provides care for a membership of over 65,000 patients in our modern suburban facilities. Inpatient care is provided at a 900-bed Tufts affiliated teaching hospital.

Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118 or call (413) 594-3111.

Medical West
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

OB/GYN - BC/BE to join respected multi-specialty group in medium-sized mid-western college community. Busy Ob/Gyn practice seeking to enhance current services in market of 90,000 adjacent to metropolitan area and medical schools. Excellent benefits and competitive salary leading to partnership opportunity. Please contact Gerald Keasling, M.D., Fremont Medical Associates, 2350 North Clarkson St., Fremont, NE 68025 or call 402/721-3133.

MATERNAL-FETAL MEDICINE

The Allentown Hospital–Lehigh Valley Hospital Center, an 830-bed tertiary care center in eastern Pennsylvania, is seeking a physician to direct the maternal-fetal service (including Level II ultrasound and antenatal fetal diagnostic procedures) and provide leadership and medical education in maternal-fetal medicine. Applicants should be board-certified or active candidates for certification in maternal-fetal medicine.

TAH–LVHC sponsors a fully accredited OB/GYN Residency Program and is a clinical campus of Hahnemann University in Philadelphia, where the successful candidate will be eligible for faculty appointment. The hospital is a referral center for a population of close to one million people and is a Level III Perinatal Center; of over 3,300 deliveries performed last year, 42% were designated high-risk. The department is supported by a 20-bed Level III NICU and also provides a full range of services to women with subspecialists in both reproductive endocrinology and gynecologic oncology. (Our current maternal-fetal subspecialist, who is our department Chair, will be leaving at the end of the year.)

The hospital is located in Allentown, a prosperous and desirable area with excellent schools and diverse cultural and recreational opportunities, one hour north of Philadelphia and one and one-half hours west of New York City.

Please submit a curriculum vitae, in confidence, to: **Headley S. White, Jr., M.D., Sr. V.P. Medical and Academic Affairs, c/o HealthSearch, 50 College Drive, Suite 325, Allentown, PA 18104, (215) 778-7993.** Equal Opportunity Employer.

OB/GYN CHIEF

The Department of Gynecology and Obstetrics at The Union Memorial Hospital is currently accepting applications for the position of Chief of Gynecology and Obstetrics and director of a fully accredited residency training program. Union Memorial is a 350 bed teaching hospital in Baltimore, Maryland. Applicants must be board certified in OB/GYN with subspecialty boards preferred, have administrative, clinical, teaching, and research experience. For confidential consideration, please send CV to:

Marcella Roenneburg, M.D.
The Union Memorial Hospital
201 E. University Parkway
Baltimore, Maryland 21218

OBSTETRICIAN/GYNECOLOGIST

Opportunity for a Board Certified/Board Eligible OB/GYN physician to join a thriving practice in existence for over 20 years. This group recently limited their practice to gynecology. However, they are seeking an associate desirous of performing both OB and GYN with plans to add another OB/GYN within one year. Call coverage will be provided. Teaching opportunities are also available in conjunction with Mercy hospital based Family Practice Residency Program.

This pracice offers a generous guaranteed salary. Excellent benefits are offered including malpractice, family health, and disability insurance, overhead expenses, moving expenses, and much more!

This area of the Great Lakes region offers a host of social, cultural, recreational, and sporting activities.

For further details about this exciting practice opportunity, please contact or forward C.V. to: Wendy Tossell, Physician Development Manager, Mercy/St. Charles Hospital, 2600 Navarre Avenue, Oregon, Ohio 43616 (419) 698-7645.

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

Seeking a BC/BE Obstetrician/Gynecologist to provide outpatient care in our Pleasanton medical office building and inpatient care at the Kaiser Hospital/Walnut Creek. Position involves supervision of NPs. Academic affiliation available. Enjoy easy access to the recreational and cultural activities of the San Francisco Bay area. We offer competitive salary and an excellent benefits package including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Patrick Golden, M.D., The Permanente Medical Group, Inc., 5600 Stoneridge Mall Road, Pleasanton, CA 94588. EOE

KAISER PERMANENTE
Good People. Good Medicine.

GEORGIA

Excellent opportunity for Board Certified/Board Eligible OB/GYN to join 38-member, multispecialty group. Practice offers established referral base with tremendous growth opportunity. Just one hour from Atlanta, Georgia. Guaranteed minimum $125,000 salary plus benefits, and 1 in 3 call. Send CV or call:

Brad Peters
JACKSON AND
COKER, INC.
115 Perimeter
Center Place
Suite 380 AJOGO
Atlanta, GA 30346
1-800-544-1987

JACKSON AND COKER

The **Michael Reese Health Plan,** a large well-establisned HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching. All specialty facilities available.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Alfred Kendrick, M.D.
Medical Affairs
MICHAEL REESE HEALTH PLAN
2545 King Drive, Chicago 60616
or call (312) 808-4551

Michael Reese Health Plan

an equal opportunity employer m/f/h

PERINATAL ASSOCIATES OF NORTHERN CALIFORNIA

Are you a clinical perinatologist? We are three perinatologists and one geneticist-OB/GYN looking for one more perinatologist who: desires superior environment for patient care, in a private, modern, level 3 facility doing 6,000 deliveries a year with superior perinatal outcome; likes personal, direct hands-on patient care; needs PUBS, IUTxF, CVS and complicated pregnancies, level 2 ultrasound; demands partners, not superiors; wants to live in clean, warm, not humid, snow-free (but 60 minutes from skiing) Sacramento, California; feels one's family has priorities, and is committed to the private practice of medicine. If really interested call (916) 733-1755, extension 2773.

Hanns C. Haesslein, M.D.
Perinatal Associates
of Northern California
5301 F. Street
Sacramento, California 95819

OBSTETRICIAN/ GYNECOLOGIST

Opportunity for a qualified OB/GYN physician to join the staff of the Medical Center of Delaware beginning July 1, 1991.

Responsibilities include treating patients in four area clinics and supervision of residents.

Generous salary and benefits package includes Professional Liability insurance, vacation, health insurance coverage, dental and prescription plans. Newark, Delaware is in close proximity to Philadelphia and Baltimore, sports, cultural events, theatre, and shopping.

Please direct inquiries and C.V. to:

Medical Center of Delaware
c/o Garrett H.C. Colmorgen, M.D.
Obstetrics and Gynecology
Maternal/Fetal Medicine
4755 Ogletown-Stanton Road
P.O. Box 6001
Newark, DE 19718

REPRODUCTIVE ENDOCRINOLOGY

The Department of Obstetrics and Gynecology at Truman Medical Center-University of Missouri at Kansas City seeks a board certified/board eligible sub-specialist to direct the Division of Reproductive Endocrinology. This position entails clinical and didactic teaching of both residents and medical students, participation in current projects and development of new areas of research. The candidate may assume a large office-based practice of subspecialty patients which offers a full range of assisted reproductive technologies. The hospital offers a very generous benefit package. Salary, hospital and university appointments will be commensurate with experience and qualifications.

Please send resume to:

Marilyn R. Richardson, M.D.
Director, Division of
Reproductive Endocrinology
Truman Medical Center
2301 Holmes Street
Kansas City, Missouri 64108

OBSTETRICIANS & GYNECOLOGISTS

MOUNT SINAI SCHOOL OF MEDICINE

Affiliation has excellent opportunities at Elmhurst Hospital Center serving western Queens community. Board certified/Board eligible candidates will join team-oriented approach providing healthcare to challenging patient mix in culturally diverse environment, located minutes from midtown Manhattan. Responsibilities include primary clinical care; routine/high-risk obstetrics; resident, fellow, and student teaching.

Academic rank granted by School of Medicine based on individual credentials. Excellent salary and benefits package. Participation in successful physician private practice plan. Send c.v. or call: **Jasmin Moshirpur, MD, Director of Obstetrics & Gynecology/Associate Dean-Elmhurst Affairs, Room #C8-13, (718) 830-1340/1348.**

MOUNT SINAI SERVICES

MSM SCHOOL OF MEDICINE OF THE CITY UNIVERSITY OF NEW YORK

Elmhurst Hospital Center
79-01 Broadway
Elmhurst, NY 11373
An equal opportunity employer

KAISER PERMANENTE

OBSTETRICIAN-GYNECOLOGIST

The Permanente Medical Group, Inc., of San Jose, California is looking for BC/BE physicians for immediate openings in the OB/GYN Department. Positions available either at the main facility in San Jose, or at satellite medical offices in Gilroy. The young, dynamic, department is still growing and currently has seventeen physicians and two nurse practitioners. Our outstanding benefits package includes full coverage malpractice insurance. San Jose and Gilroy share excellent access to Bay Area cultural and recreational activities.

Recent salary advances allow extremely competitive compensation starting with the first year of employment.

For more information, please contact:

Jerome L. Nehemiah, M.D.
Chief, Department of Ob/Gyn
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119
or telephone: 408-972-6180 (collect)

OB/GYN SINGLE-SPECIALTY GROUP

ILLINOIS

OB/GYN needed for an outstanding community of 100,000. Modern office is attached to a progressive, 325-bed hospital. Group performs over 1,000 deliveries per year. New Obstetric unit has 9 LDRP's. State-of-the-art equipment inclues lasers, CT scanner, and MRI.

Community has excellent schools and a strong, growing economy. Enjoy this safe, clean living environment. Send CV or call:

Greg E. Flowers
JACKSON AND COKER, INC.
115 Perimeter Center Place
Suite 380 AJOGO
Atlanta, GA 30346
1-800-544-1987

JACKSON AND COKER

Perinatology Director Sarasota, Florida

Develop a perinatology service in an established 34-bassinet, Level III NICU for 850+ bed, full service, public, not-for-profit hospital. Supported by 2 neonatologists and 23 active obstetricians delivering over 3K babies a year. The hospital is only ten minutes from prime Florida Gulf Coast beaches in a culturally sophisticated community of 260K. Serve as primary consultant on all high risk cases. One-year guarantee as an independent contractor, stipend, superb benefits, and relocation assistance. Proven Director or Assistant Director with strong interpersonal skills preferred. Contact Robin Walker, Tyler & Company, 9040 Roswell Road, Suite 550, Atlanta, Ga 30350 or call 404-641-6411/800-223-3659.

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting for positions in our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

REPRODUCTIVE ENDOCRINOLOGIST

• SAN JOSE •

We are currently seeking a Reproductive Endocrinologist to establish and run a reproductive endocrinology subdivision in the department. Duties would include the full spectrum of reproductive endocrinology, outpatient and surgical management of infertility. Fellowship certification would be preferred. The department currently has 17 physicians and is still growing. We offer a generous benefits package including full coverage malpractice insurance. San Jose has beautiful weather and excellent access to Bay Area recreational and cultural activities.

For more information, please contact: Jerome L. Nehemiah, M.D., Chief, Department of OB/GYN, The Permanente Medical Group, Inc., 260 International Circle, Dept. AJOBG, San Jose, CA 95119, or call collect (408) 972-6180. EOE

KAISER PERMANENTE

Good People. Good Medicine.

OB-GYN

BC/BE OB-GYN sought for full service, 260-bed community hospital in San Francisco's dynamic South of Market area.

- Excellent opportunity to join busy, growing practice.
- Competitive salary and benefits package.

ST. LUKE'S HOSPITAL

Send C.V. to:

Walter Kopp
St. Luke's Hospital
3555 Army Street
San Francisco, CA 94110
(415) 641-6543

Full Time Faculty Positions

The Department of OB/GYN at Temple University Health Science Center is seeking Board Certified or Board Eligible Obstetrician/Gynecologists in the following areas:

MATERNAL-FETAL MEDICINE
GYNECOLOGIC ONCOLOGY
REPRODUCTIVE ENDOCRINOLOGY
GENERAL OBSTETRICS & GYNECOLOGY

Clinical and academic activities are integrated in a department providing tertiary services. Newly opened Temple University Hospital houses a modern Labor and Delivery Suite and large Neonatal Intensive Care Unit. Candidates should have an active interest in Research, Clinical Practice and Resident and Medical Student Education. Salary and academic rank commensurate with experience. Submit C.V. to: **E. Albert Reece, M.D., Chairman, Dept. of OB/GYN, TEMPLE UNIVERSITY HEALTH SCIENCE CENTER, Broad & Ontario Sts., Philadelphia, PA 19140.** Equal Opportunity Employer.

UNIVERSITY

Choose
TEMPLE

Join Our Team

Here's your opportunity to relocate to our small, friendly community high in the beautiful White Mountains of New Hampshire with year-round recreational activities at your doorstep.

Weeks Memorial Hospital, in Lancaster, New Hampshire, offers you a family centered atmosphere in a 49-bed acute-care facility, big enough to be exciting, but small enough to give individual attention.

We are seeking an **Obstetrician/Gynecologist** to join our team of family practice physicians and internists. Put your personal satisfaction and career development first.

Call collect or send your resume to: Administration, Weeks Memorial Hospital, RR 1, Box 8, Lancaster, N.H. 03584.

Weeks Memorial Hospital

603/788-4911 ext. 303

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

BC/BE Obstetrician/Gynecologist sought to join small congenial clinic setting within established pre-paid multispecialty group. Practice consists of general obstetrics and gynecology, high risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser are welcomed. Association with resident staff and teaching opportunities available.

Kaiser Permanente offers competitive salaries and excellent benefits including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE



KAISER PERMANENTE
Good People. Good Medicine.

UTMB **University of Texas Medical Branch Galveston, Texas**

The Department of Obstetrics and Gynecology is currently recruiting for:

Gynecologic Oncologist

- Board eligibility or certification in gynecologic oncology required.
- Proven record of research and commitment to an academic career.
- Academic rank and salary commensurate with experience and qualifications.

Curriculum Vitae should be sent to:

Garland D. Anderson, M.D.
Jennie Sealy Smith Professor & Chairman
Department of Obstetrics & Gynecology
The University of Texas Medical Branch
Galveston, Texas 77550-2776

UTMB is an equal opportunity M/F/V/H affirmative action employer. UTMB hires only individuals authorized to work in the United States.

YALE UNIVERSITY MEDICAL SCHOOL
Department of Epidemiology and Public Health
Training Program in Perinatal Epidemiology

United States citizens and permanent residents are invited to apply for predoctoral and postdoctoral fellowships in perinatal epidemiology.

Predoctoral fellows will prepare for the Ph.D. degree in Chronic Disease Epidemiology with a specialty (course work, tutorials and thesis) in perinatal epidemiology. Applicants must meet matriculation requirements of Yale Graduate School.

Postdoctoral fellows, may be physicians, must have a specialty in obstetrics, pediatrics, or perinatology, or may hold other doctoral degrees. The fellowship is a two-year program with an option to graduate with the MPH degree if appropriate matriculation, course, thesis and tuition requirements are met.

For more information, or to submit CV and names of referees, write

Michael B. Bracken
Professor of Epidemiology
Yale University School of Medicine
60 College St., New Haven, CT 06510

Yale University is an Affirmative Action/Equal Opportunity Employer

---



Lynn Community Health Center *is an independently incorporated out-patient care facility with 110 staff and providing 60,000 patient visits per year.*

OBSTETRICIAN/ GYNECOLOGIST

BOSTON AREA

Expanding community health center seeking board certified or board eligible OB/GYN interested in working in a multi-speciality community health center providing care to a diverse patient population. Located in a residential area approximately twelve miles north of Boston. Excellent salary and benefit package offered.

Patricia Bongiorno
Lynn Community Health Center
P.O. Box 526, Lynn, MA 01903

OBSTETRICIAN/ GYNECOLOGIST

Immediate opening for a Board Certified/Eligible physician to work with 5 others at a well-established, growing HMO. Rochester Medical Group is a multispecialty medical group practice serving 45,000 patients at multiple sites. Clinical faculty appointment at the University of Rochester is available with this position. Competitive salary/ benefits with opportunities for research and resident teaching. Send resume to:

Patricia Hosking
Rochester Medical Group, PC
1800 Hudson Avenue
Rochester, NY 14617

Maternal Fetal Medicine Fellowshi

Department of Obstetrics and Gynecolog
Southern Illinois Universit
School of Medicin
Springfield, Illinoi

The Department of Obstetrics and Gynecology at Southern Illinoi University School of Medicine, Springfield, Illinois has one first yea Fellowship position available in Maternal Fetal Medicine beginnin July 1, 1991. This is a two year accredited Fellowship program i which both clinical and research training are emphasized. Clinica material is drawn from the 3000 deliveries/year at St. John' Hospital Perinatal Center where fellows participate in a supervise in-depth experience in management and treatment of medical an obstetrical complications with emphasis on antenatal diagnosti procedures and ultrasound techniques. This flexible prograr incorporates a minimum six month period of full time research an accommodates various clinical/research objectives based on th fellow's individual goals. Fellows are encouraged to present paper and to represent the Maternal Fetal Medicine Division at nationa scientific meetings.

Application requests should be sent to:
Robert C. Kaufmann, M.D., Department of OB/GYN, Southern Illinoi University School of Medicine, P.O. Box 19230, Springfield, IL 62794. Phone: (217) 782-8240

SIU is an AA-EEO Employer

DIRECTOR OF DEPARTMENT OF OBSTETRICS and GYNECOLOGY

St. Luke's/Roosevelt Hospital Center, a 1,315 bed teaching hospital on Manhattan's West Side, affiliated with the Columbia University College of Physicians and Surgeons, invites nominations and applications for a Director of the Department of Obstetrics and Gynecology. The Director is responsible for the clinical, academic and research programs at both the St. Luke's and Roosevelt sites which together serve a catchment populations of over 500,000 as well as a large referral base from well beyond this local community.

The successful candidate must qualify as a senior faculty member at Columbia University with demonstrated clinical and academic achievement. Administrative and leadership skills will also be required in order to maintain and foster the department's current accomplishments.

Curricula vitae will be considered in strictest confidence.

Sigurd Ackerman, M.D.
Chairman, Search Committee for a Director
of the Department of Obstetrics and Gynecology
ST. LUKE'S/ROOSEVELT HOSPITAL CENTER
114th Street and Amsterdam Avenue
New York, N.Y. 10025
An Equal Opportunity Employer M/F

ST. LUKE'S\ROOSEVELT
Hospital Center

OB/GYN- SOUTHWESTERN MICHIGAN

OB/GYN to join another OB/GYN and a nurse-midwife in clinic practice. Average 600 deliveries per year. Opportunity has an excellent mix of GYN Pathology.

330-bed facility in a service area of 215,000 has 4 LDR's, 24-hour, in-house Anesthesia (Epidural Service), and a Level II nursery. Community is located on Lake Michigan and is 90 minutes from Chicago in Southwestern Michigan.

$150,000 base salary. Production bonus may increase income by another $50,000 to $100,000 the first year. Send CV or call:

Ken Sammut
JACKSON AND COKER, INC.
115 Perimeter Center Place
Suite 380 AJOGO
Atlanta, GA 30346
1-800-544-1987

JACKSON AND COKER

UNIVERSITY OF MIAMI
SCHOOL OF MEDICINE
DEPARTMENT OF OBSTETRICS & GYNECOLOGY

ASSISTANT/ASSOCIATE PROFESSOR OF CLINICAL OBSTETRICS & GYNECOLOGY

Opportunity available for M.D. who is board-certified or board-eligible in Maternal Fetal Medicine. Special interest in infectious disease is desirable but not required.

Applicant must be licensed to practice medicine in State of Florida, and have sufficient clinical experience to qualify for a full time faculty appointment.

Salary will be commensurate with training and experience. Compensation includes excellent fringe benefit and financial incentive plan.

Apply to:

Mary J. O'Sullivan, M.D.
Professor
Obstetrics & Gynecology, R-136
University of Miami
P.O. Box 016960
Miami, Florida 33101

AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

LAS VEGAS—Seeking highly qualified board certified/eligible Ob/Gyn physicians to join our 60 physician multi-specialty group. Practice in America's Vacationland—mountain sports, water sports, world class entertainment. Enjoy affordable housing, no state income tax, excellent schools/colleges. High starting salaries, plus bonuses, no investment required. Benefits include malpractice insurance, paid vacation, relocation expenses, health and life insurance, retirement program, continuing medical education, dues, subscriptions, etc. Top tertiary hospitals.

If interested, call me at (702) 877-8638 or send your Curriculum Vitae to: Jerry Reeves, M.D., President, Southwest Medical Associates, P.O. Box 15645, Las Vegas, NV 89114.

FACULTY POSITION UNIVERSITY OF WASHINGTON

A University of Washington junior faculty position is available in the Gynecology Division in the Department of Obstetrics and Gynecology. Patient responsibility will involve both gynecologic outpatient and inpatient care. The individual will spend 60% time in clinical practice and the remaining time conducting medical student teaching, resident teaching and clinical research. Applicants must be board eligible or certified in obstetrics or gynecology. Fellowship or research experience is desirable. The position is immediately available.

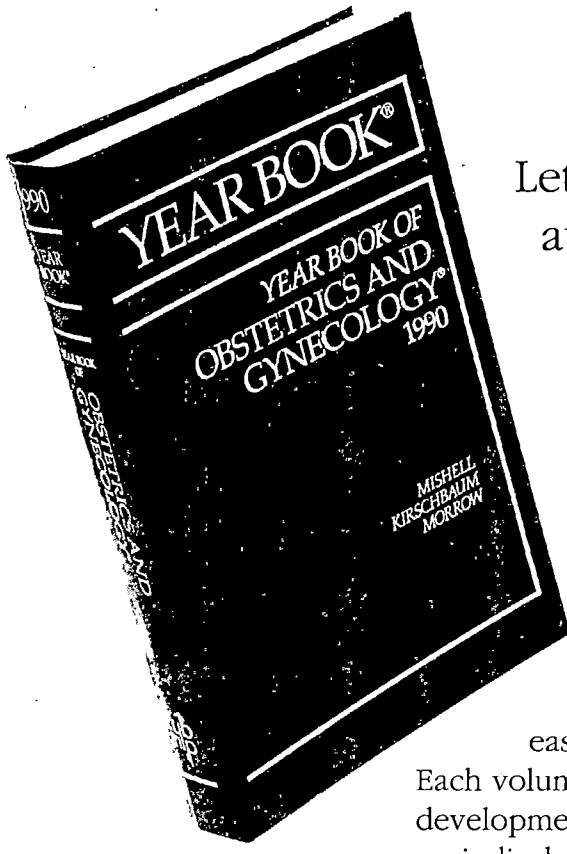
Please send a curriculum vitae to:

David A. Eschenbach, M.D.
Department of Obstetrics and Gynecology, RH-20
University of Washington,
Seattle, WA 98195

An equal opportunity/affirmative action employer.

PERINATAL PATHOLOGIST AT NORTHWESTERN UNIVERSITY MEDICAL SCHOOL. The Department of Pathology of Northwestern University Medical School seeks applicants with subspecialty training and experience in perinatal pathology, for a full-time faculty position. The academic level of appointment and salary will be commensurate with prior experience and scholarly accomplishment. The Department is developing a perinatal program in conjunction with the Department of Obstetrics and Gynecology. The Prentice Women's Hospital of McGaw Medical Center currently has 5000 deliveries/year and has a high risk pregnancy program which serves greater metropolitan Chicago, the State of Illinois and adjacent States. Children's Memorial Hospital is also part of the Medical School and Department's teaching program. The Department of Pathology has active residency, fellowship, and graduate student training programs. The successful candidate will be skilled in relevant aspects of perinatal pathology, teaching, and conduct either clinical or basic research. Interested candidates should submit a curriculum vitae and 3 letters of reference by December 31, 1990 to: Dr. Larry Sherman, Department of Pathology, Northwestern Memorial Hospital, Superior Street and Fairbanks Court, Chicago, IL 60611.

Northwestern University is an Affirmative Action/Equal Opportunity Employer. Hiring is contingent upon eligibility to work in the United States.



Let our eminent medical authorities guide you through major new clinical advances

Introducing the

Year Book of Obstetrics & Gynecology®

This time-saving guide book makes it easy for you to keep current in your field. Each volume represents the year's most significant developments as reported in more than 800 journals and periodicals published worldwide. These selections are condensed to roughly a page in length, so you get all essential facts at a glance.

More importantly, the distinguished physician editors provide thoughtful, probing commentaries that explore the significance of each article while evaluating its practical implications for day-to-day practice.

Editors: Daniel R. Mishell, Jr., M.D.; Thomas H. Kirschbaum, M.D.; and C. Paul Morrow, M.D. **Contributing Editor**: William H. Hindle, M.D.

These books are published annually. For the convenience of its customers, Year Book enters each purchaser as a subscriber to future volumes and sends annual announcements of each volume approximately two months before publication. The new volume will be shipped upon publication unless you complete and return the cancellation notice attached to the announcement and it is received by Year Book within the time indicated (approximately 20 days after your receipt of the announcement). You may cancel your subscription at any time. The new volume may be examined on approval for 30 days and returned for full credit. Year Book will remove your name as a subscriber. Return postage is guaranteed by Year Book to the Postal Service.

To Subscribe:

Simply fill out this coupon and send it to us at the address shown.

Or,

for faster service, call our TOLL-FREE number:

1-800-426-4545

Prepaid orders are shipped postage free; add $3.50 per order to cover handling. Other orders will be billed a shipping and handling charge. IL, MA, TN residents will be billed appropriate sales tax. Prices are quoted in U.S. dollars. Canadian orders payable in U.S. funds. All prices subject to change without notice.

[X] *YES!*

I know it's vital that I stay informed. Please rush me the most recent issue of the *Year Book of Obstetrics & Gynecology* (5-90) for $54.95.

❒ Payment Enclosed ❒ Please bill me ❒ Charge my Amex/Visa/MasterCard (circle one)

Signature

Account Number/Expiration Date

Name

Address

City/State/Zip

Telephone XX9

Mail to:

Mosby-Year Book Inc.

Order Processing, 11830 Westline Industrial Dr.

St. Louis, MO 63146

INDEX TO ADVERTISERS

Akron City Hospital
Opportunity Available 46A

Aspen Medical Group
Opportunity Available 46A

Bowman Gray School of Medicine
Opportunity Available 47A

Bridgeport Hospital
Opportunity Available 47A

Burroughs Wellcome Company
Zovirax 13A,14A

Christian Hospital Medical Center
Opportunity Available 47A

Columbia Hospital for Women
Opportunity Available 48A

Copley Hospital
Opportunity Available 48A

Dekalb Medical Center
Opportunity Available 49A

Dugger, David E., M.D.
Opportunity Available 48A

Eastern Virginia Medical School
Opportunity Available 51A

Elko Regional Medical Center
Opportunity Available 49A

Fish Memorial Hospital
Opportunity Available 49A

Fremont Medical Associates
Opportunity Available 52A

G.D. Searle & Company
Demulen 1/35 ..... 29A,30A,31A,32A

Group Health Plan
Opportunity Available 49A

HealthSearch
Opportunity Available 50A,52A

Jackson and Coker, Inc.
Opportunity Available 50A,51A,53A,55A,58A

Kaiser Permanente
Opportunity Available 45A

Loyola University Chicago
Opportunity Available 50A

Lynn Community Health Center
Opportunity Available 57A

Mead Johnson Laboratories
Ovcon 35 ..... 9A,10A,43A,44A

Marshfield Clinic
Opportunity Available 51A

Medical Center of Delaware
Opportunity Available 54A

Medical East
Opportunity Available 52A

Medical West
Opportunity Available 53A

Michael Reese Health Plan
Opportunity Available 54A

Mercy/St. Charles Hospital
Opportunity Available 53A

Mount Sinai Services
Opportunity Available 55A

Northwestern University
Opportunity Available 59A

Ortho Pharmaceutical Corporation
Ortho-Novum 7/7/7 ..... Third Cover,Fourth Cover

Osler Institute, The
Course 62A

Perinatal Associates of Northern California
Opportunity Available 54A

Permanente Medical Group, The
Opportunity Available 53A,55A,56A,57A

Permanente Medical Group Northern California
Opportunity Available 20A

Planned Parenthood of Tomplins County
Opportunity Available 46A

Rochester Medical Group, PC
Opportunity Available 58A

Roerig
Unasyn 37A,38A,39A,40A,41A

Southern Illinois University School of Medicine
Opportunity Available 58A

Southwest Medical Associates
Opportunity Available 59A

St. Luke's Hospital
Opportunity Available 56A

St. Luke's/Roosevelt
Opportunity Available 58A

St. Vincent Medical Center
Opportunity Available 45A

Stuart Pharmaceuticals
Cefotan 5A,6A

Syntex Laboratories, Inc.
Femstat Prefill ..... Second Cover,1A,2A
Synarel 33A,34A,35A,36A

Temple University
Opportunity Available 56A

Truman Medical Center
Opportunity Available 54A

Tyler & Company
Opportunity Available 55A

Union Memorial Hospital, The
Opportunity Available 53A

United State Air Force
Recruitment 17A

University of Chicago
Course 40A

University of Miami
Opportunity Available 59A

University of Texas Medical Branch
Opportunity Available 57A

University of Texas Southwestern Medical Center at Dallas
Opportunity Available 46A

University of Miami
Opportunity Available 59A

University of Washington
Opportunity Available 59A

Weeks Memorial Hospital
Opportunity Available 56A

Yale University School of Medicine
Opportunity Available 57A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

The Osler Institute

Obstetrics and Gynecology Review Course

February 2-7, 1991 – Los Angeles
November 25-30, 1991 – Chicago

Now, your course with case-list reviews and mock oral exams

OBJECTIVES
- Increase basic knowledge and clinical skills in ob-gyn.
- Assist residents and fellows to organize study
- Prepare exam candidates to pass their tests.
- Provide practicing gynecologists with an update

METHODS
- HOME STUDY MATERIALS with questions and answers
- SEMINAR with projection slides and syllabus
- LABORATORY with microscopic slides
- PRACTICE EXAMS with oral and written parts

*"The faculty was outstanding. The most pleasant thing was learning a tremendous amount, not only from world-famous authorities but from people who are relatively unknown as well."**

TOPICS

Clinical Science
- Surgical Anatomy
- Embryology
- Radiology
- Genetics
- Teratology
- Anesthesiology
- Antibiotics

Pathology
- Vulva
- Vagina
- Cervix
- Endometrium
- Myometrium
- Ovary
- Placenta

Reproductive Endocrinology
- Contraception
- Infertility
- Amenorrhea
- Abnormal Bleeding
- Androgen
- Prolactin
- Menopause

Oncology
- Chemotherapy
- Vulva and Vagina
- Cervix
- Uterus
- Ovary
- Trophoblast
- Breast

Benign Gynecology
- Infectious Diseases
- Urinary Retention
- Urinary Incontinence
- Pediatric Gynecology
- Sexual Assault
- Ectopic Pregnancy
- Endometriosis

Maternal and Fetal Medicine
- Pharmacology
- Diabetes
- Hypertension
- Hematology
- Antepartum Testing
- Premature Labor
- Birth Trauma

FACULTY

Theresa Abboud, M.D.
Univ. of Southern California

Arieh Bergman, M.D.
Univ. of Southern California

Carolina Braga, M.D., B.S.
Univ. of Calif., San Francisco

John P. Curtin, M.D.
Univ. of Southern California

Edward Hill, M.D.
Univ. of Calif., San Francisco

Karen Ireland, M.D.
Oregan Health Science Univ.

Conley Lacey, M.D.
Scripps Clinic, La Jolla, CA

David Plourd, M.D.
Santa Clara Valley Med. Ctr.

C. Bethan Powell, M.D.
Washington University

Jeffrey Stern, M.D.
Univ. of Calif., San Francisco

Nelson Tang, M.D., Ph.D.
Stanford University

Wendell Wall, M.D.
University of North Dakota

Peter Watson, M.D.
Oregan Health Science Univ.

David R. Witt, M.D.
Kaiser Med. Ctr., San Jose, CA

*"Accommodations were comfortable...."**

GOALS AND LOCATION: This course for residents and practicing gynecologists is a comprehensive review and update offered in the winter, spring and fall. The fall course is more clinically oriented. Home study questions are sent upon registration. The course includes lectures with syllabus as well as an opportunity to purchase an unlimited number of optional extra one-on-one sessions with faculty to review your case lists or do mock oral exams. Past participants recommend repeating the course for half price immediately before exams. The course hotel is chosen in competetive bidding to achieve the best combination of bargain rates and excellent study environment.

*"...and those little extras...."**

LOWEST AIR FARES: Please call toll-free 1-800-548-8185 for group discounts.

*"...the most education for the money."**

FEES:
- Practicing Gynecologists: $720
- Residents and Fellows: $480
- Repeating course within 3 years: $360
- Case-list reviews and mock oral exams (half hour sessions): $ 70
- Private mock oral exams: $100
- Optional extra day Dec. 1 (more questions, answers and pathology lab): $15(
- **Add 10% for within 10 days of the course.**
- Attendees not in course hotel add $20/day
- A deposit of $50 will reserve your position

"...home study material was extremely helpful."

CANCELLATIONS: Refunds subject to $5 fee, will be made until the seminar begins

*"...remarkably complete and pleasant."**.

INFORMATION:
Joseph H. Selliken, Jr., M.D.
The Osler Institute
1094 Dawn Lane, P.O. Box 2218
Terre Haute, IN 47802
(800) 356-7537 or (812) 299-5658

* *Comments by participants.*

Limited Enrollment: OBSTETRICS AND GYNECOLOGY REVIEW REGISTRATION

Name _____

Address _____

City/State/Zip _____

Phone _____

Mail today to:

1094 Dawn Lane, Dept. 12
P.O. Box 2218
Terre Haute, IN 47802

For: ☐ February 2-7, 1991 – Los Angeles
☐ November 25-30, 1991 – Chicago
☐ Check enclosed for $_____
☐ Please send more information

ORTHO-NOVUM® 777 Tablets

norethindrone/ethinyl estradiol

Smooth Sailing!



Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28 day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

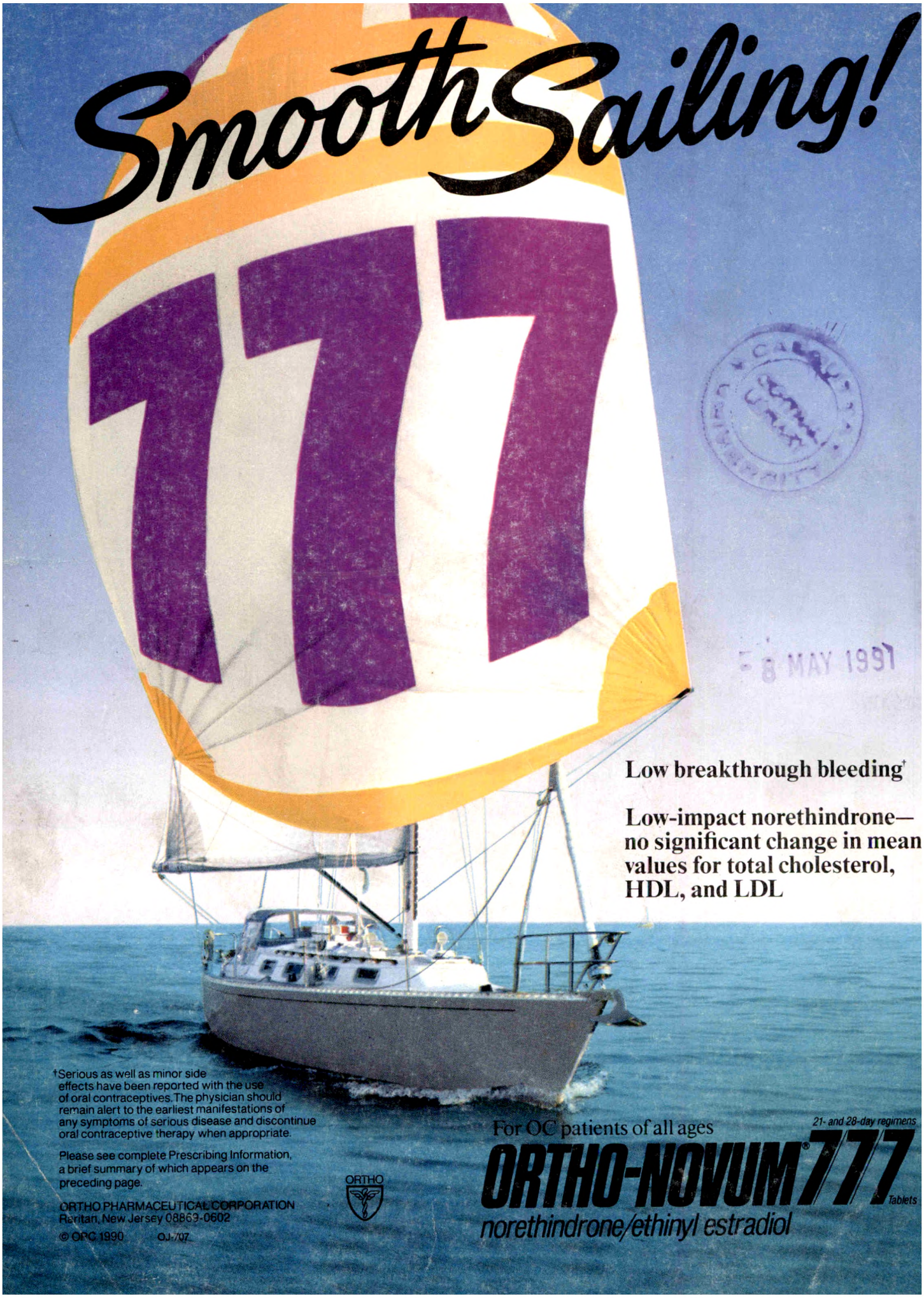
Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

ORTHO PHARMACEUTICAL CORPORATION
Raritan, NJ 08869-0602

ORTHO


Smooth Sailing!
777
Low breakthrough bleeding†
Low-impact norethindrone—no significant change in mean values for total cholesterol, HDL, and LDL
†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.
Please see complete Prescribing Information, a brief summary of which appears on the preceding page.
ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602
© OPC 1990 OJ-707
ORTHO
For OC patients of all ages
21- and 28-day regimens
ORTHO-NOVUM® 777 Tablets
norethindrone/ethinyl estradiol

December 1990
in two parts, part 2
volume 163, *number* 6

Aug 12 92

SUPPLEMENT TO

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

PHARMACOKINETICS OF ORAL CONTRACEPTIVE STEROIDS AND DRUG INTERACTION

Salzburg, Austria
September 14-16, 1989

Editor
Prof. Dr. phil. nat. Herbert Kuhl
Department of Obstetrics and Gynecology
J. W. Goethe University
Frankfurt am Main, Germany



Published by
MOSBY-YEAR BOOK, INC.
St. Louis, Missouri 63146-3318

ISSN 0002-9378

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1990 by Mosby-Year Book, Inc.

PHARMACOKINETICS OF ORAL CONTRACEPTIVE STEROIDS AND DRUG INTERACTION

Salzburg, Austria
September 14-16, 1989

Editor
Prof. Dr. phil. nat. Herbert Kuhl
Department of Obstetrics and Gynecology
J. W. Goethe University
Frankfurt am Main, Germany

Mosby Year Book

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1990 by Mosby–Year Book, Inc.

Contents

December Part 2
1990

Introduction 2113
Prof. Dr. Herbert Kuhl, PhD, *Guest Editor*

SESSION I. PHARMACOKINETICS OF ORAL CONTRACEPTIVE STEROIDS

Chairman: K. Fotherby

Pharmacokinetics of ethinyl estradiol and mestranol 2114
Joseph W. Goldzieher, MD, and Steven A. Brody, MD
Houston, Texas

Interindividual and intraindividual variations as well as ethnic factors affect the pharmacokinetics of ethinyl estrogens.

Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women 2120
Wilhelm Kuhnz, PhD
Berlin, West Germany

During the long-term administration of the synthetic progestins levonorgestrel and gestodene, the observed steady-state concentrations in serum were higher than anticipated from single-dose pharmacokinetics.

Chairman: J. W. Goldzieher

Pharmacologic and pharmacokinetic characteristics of norgestimate and its metabolites 2127
John L. McGuire, PhD, Audrey Phillips, PhD, DoWon Hahn, PhD, Edward L. Tolman, PhD, Soledad Flor, PhD, and Michael Edwin Karfrissen, MD, MSPH
Raritan, New Jersey

Data suggest that 17-deacetyl norgestimate contributes to the pharmacology of its parent compound, norgestimate.

(Contents continued on page 4A)

Vol. 163, No. 6, Part 2, December 1990. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send change of address to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 4351. Annual subscription rates for 1991: domestic, $86.00 for individuals and $150.00 for institutions. Printed in the U.S.A.
Copyright © 1990 by Mosby–Year Book, Inc.

Contents continued from page 3A

Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin 2132
Willem Bergink, PhD, Roel Assendorp, MSc, Lenus Kloosterboer, PhD, Wim van Lier, MSc, Gerrit Voortman, MSc, and Ingelise Qvist, MD
Oss, The Netherlands, and *Horsens, Denmark*

The pharmacokinetics of desogestrel (0.150 mg) and ethinyl estradiol (0.030 mg) and the binding affinities of progestogens derived from norethisterone for sex hormone–binding globulin were estimated.

SESSION II. GASTROINTESTINAL METABOLISM OF ORAL CONTRACEPTIVE STEROIDS

Chairman: J. Hammerstein

Gastrointestinal metabolism of contraceptive steroids 2138
David J. Back, PhD, Steven Madden, BSc, and Michael L'E. Orme, MD
Liverpool, England

The article summarizes both in vitro and in vivo studies on gut wall metabolism of the contraceptive steroids ethinyl estradiol, desogestrel, and norgestimate.

Factors affecting the enterohepatic circulation of oral contraceptive steroids 2146
Michael L'E. Orme, MD, and David J. Back, PhD
Liverpool, England

The enterohepatic circulation of contraceptive steroids applies only to the estrogens, particularly ethinyl estradiol, and clinical and laboratory studies have not found convincing evidence of its importance.

1991 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)* Canada	Mexico	Region 1	Region 2	Region 3
Institutions†	$150.00	$182.00	$215.00	$223.00	$238.25	$290.50	$342.50
Individuals‡	$ 86.00	$118.00	$181.00	$159.00	$174.25	$226.50	$278.50
Students, residents‡	$ 40.00	$ 72.00	$105.00	$113.00	$128.25	$180.50	$232.50

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.
*Airmail breakdown—*Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia. Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.
†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.
‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.
Subscriptions may begin at any time.

Contents

SESSION III. HEPATIC METABOLISM OF ORAL CONTRACEPTIVE STEROIDS

Chairman: M. Orme

Interactions with oral contraceptives 2153
Kenneth Fotherby, PhD
London, England

The many factors that may affect the pharmacokinetics and pharmacodynamics of oral contraceptives and the interaction of the estrogen and gestagen components are considered.

Inhibition of oral contraceptive steroid–metabolizing enzymes by steroids and drugs 2159
F. Peter Guengerich, PhD
Nashville, Tennessee

The major oxidative pathway of 17α-ethinyl estradiol metabolism, 2-hydroxylation, is inhibited by several chemicals; gestodene is an inactivator in the human liver.

Formation, metabolism, and physiologic importance of catecholestrogens 2163
Peter Ball, Prof. Dr. med, and Rudolf Knuppen, Prof. Dr. Rer. Nat.
Lubeck, West Germany

Aromatic orthohydroxylation of primary estrogens, such as estradiol and ethinyl estradiol, is an important reaction in mammals.

SESSION IV. HORMONAL EFFECTIVENESS OF ORAL CONTRACEPTIVE STEROIDS

Chairman: M. Breckwoldt

Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor 2171
Michael Juchem and Kunhard Pollow, MD, PhD
Mainz, West Germany

Progestogens that are used in oral contraceptives are characterized at the level of receptors as well as at the level of high-affinity steroid-binding serum proteins.

(Contents continued on page 6A)

Contents continued from page 5A

Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: Factors influencing the steroid metabolism 2183
Claudia Jung-Hoffmann, MD, and Prof. Dr. Herbert Kuhl, PhD
Frankfurt am Main, Federal Republic of Germany

The pharmacokinetics of contraceptive steroids during treatment with low-dose oral contraceptives indicate that ethinyl estradiol and nortestosterone derivatives may inhibit steroid metabolism.

Prodrugs: Advantage or disadvantage? 2198
Jürgen Hammerstein, MD
Berlin, West Germany

The use of hormonal prodrugs instead of drugs results in minor pharmacokinetic, pharmacodynamic, and potency changes with limited clinical impact, if any.

SESSION V. CLINICAL IMPLICATIONS

Chairman: H. D. Taubert

Gastrointestinal disease and oral contraception 2204
Jürgen P. Hanker, MD
Münster, West Germany

Gastrointestinal disease may impair contraceptive protection by reducing the bioavailability of oral contraceptive steroids.

Influence of oral contraceptives on drug therapy 2208
Alexander T. Teichmann, Prof. Dr. med.
Göttingen, West Germany

Oral contraceptives can modify pharmacokinetics of various drugs mainly by inhibition of oxidative metabolism and stimulation of conjugation with acids and may also interfere with pharmacodynamics.

Oral contraception in disease states 2213
M. Breckwoldt, P. Wieacker, and F. Geisthövel
Freiburg im Breisgau, Germany

Absolute and relative contraindications to the use of oral contraceptives are presented and discussed, with special reference to long-term diseases.

Conclusion 2216
Prof. Dr. Herbert Kuhl, PhD
Frankfurt am Main, Federal Republic of Germany

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 163 *number* 6 *part* 2 DECEMBER 1990

Introduction

The purpose of an oral contraceptive is to inhibit ovulation reliably, to maintain good cycle control, and to cause minimal adverse effects. The response of individual women to a given formulation differs to a large degree. Although the pill producers and the medical boards and authorities are, in the first place, interested in average figures of efficacy, adverse effects, and mortality, women who take the pill and the practitioner who prescribes it, are faced with individual susceptibilities, responses, and side effects. For the past 30 years ago, choosing the most suitable formulation has remained for each woman a matter of experience. Undoubtedly all formulations, including low-dose preparations, are overdosed for most women, because they must suppress ovulation in all women.

The pill is a combination of two hormones that, as far as the dose is concerned, belong to the most potent drugs known. Although it has a large therapeutic margin and serious adverse effects are rare, liver damage, stroke, or thromboembolic disease during intake of an oral contraceptive is a vital question for the individual. Any attempt to individualize the prescription of oral contraceptives on a scientific basis is dependent on the pharmacokinetics of the estrogen and progestogen components, which show large variations from woman to woman and from day to day. Therefore one of the most important aims of research must be to investigate the factors that influence the pharmacokinetic properties of contraceptive steroids.

The history of the pill since the days of Pincus is characterized by not only empiricism but also by errors. The suspicion that the estrogen component is involved in the etiology of cardiovascular diseases has led to a continuous reduction in the dose, which was hampered from time to time by the assumption that there must be a threshold dose of ethinyl estradiol with respect to good cycle control (e.g., 50 μg 20 years ago or 30 μg today). In both cases reality proved it to be wrong, provided that the progestogen was chosen properly. We are still waiting for a new estrogen that has no toxic effects on the liver. The development of the progestogens was decisively influenced by the "beagle story," when derivatives of progesterone were unfoundedly suspected of causing breast cancer. Although the hepatic effects of this type of progestogen are admittedly much less than those of ethinylated nortestosterone derivatives, new courses were set for the development of the so-called "gonanes." By the same measure, as potency was increased, the dose was reduced, which was claimed to be progress because of a pretendedly less burden of hepatic metabolism. However, a comparison of the pharmacokinetics would easily demonstrate that this is very questionable. The gonanes are orally more potent because their elimination is slowed down, and the serum concentrations of new and very low-dose progestogens may reach very high values. There are some indications that the slow inactivation rate of nortestosterone derivatives is not primarily the result of a steric hindrance of enzymatic action by the ethinyl group, but rather of a direct inhibition of enzymes by the chemically activated ethinyl group.

Even though there is no doubt that contraceptive steroids in the blood are of much higher importance for the assessment of the effects and risks than the dose ingested, pharmacokinetics are of limited interest for most gynecologists and endocrinologists. One of the aims of the symposium on "Pharmacokinetics of Oral Contraceptive Steroids and Drug Interactions" was to focus attention on this topic. More than 10 years have passed since a similar workshop conference was held in Igls, Austria, in 1978, and among the 18 experts assembling in Salzburg on September 14 to 16, 1989, some participated in the meeting held 1 decade ago. Obviously, the questions and problems discussed in Salzburg in 1989 have not changed since 1978.

As with the workshop conference in Igls, Austria, in 1978, the symposium held in Salzburg in 1989 was sponsored by Organon, Munich. It was because of Mrs. Franz and Dr. Geissler (Organon GmbH, Munich) that the organizational setting and the preconditions for a successful meeting have been optimal. Moreover, the generous support from Organon GmbH (Munich) and Organon International bv (Oss, Netherlands) enabled the publication of all the papers presented at the symposium in this supplement to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

Prof. Dr. Herbert Kuhl, PhD
Guest Editor

6/0/23620

SESSION I. PHARMACOKINETICS OF ORAL CONTRACEPTIVE STEROIDS

Chairman: K. Fotherby

Pharmacokinetics of ethinyl estradiol and mestranol

Joseph W. Goldzieher, MD, and Steven A. Brody, MD
Houston, Texas

Pharmacokinetally, a 50 μg oral dose of mestranol (which itself is inactive) is bioequivalent to a 35 μg dose of ethinyl estradiol. Physiologically, mestranol ranges from 50% to 100% of the activity of ethinyl estradiol, depending on the endpoint chosen. Compounds such as these, which are metabolized with a first-pass effect and are enterohepatically recirculated, demonstrate large interindividual and intraindividual variability in their pharmacokinetics. Thus a given dose of ethinyl estradiol in one person may produce an effect equivalent to a substantially larger (or smaller) dose in another person. This wide variability confounds efforts to establish tight dose-response relationships, a point rarely considered in clinical or epidemiologic studies of these compounds. The circulating levels of ethinyl estradiol sulfates may be higher than those of free ethinyl estradiol itself. It has been thought that these sulfates represent a "reservoir" of ethinyl estradiol. Our studies show that this idea is untenable because the half-life of the sulfates is not long enough for such an effect. Differences in the pharmacokinetics of ethinyl estradiol and mestranol have been observed in studies of various populations. The reality of these group differences is affirmed by analyses of urinary metabolite patterns. (Am J Obstet Gynecol 1990;163:2114-9.)

Key words: Ethinyl estradiol, mestranol, pharmacokinetics

All oral contraceptives currently in use rely on ethinyl estrogens for their estrogenic component. The reason for this is the greatly increased pituitary-inhibiting activity relative to other estrogenic effects, that is imparted by the ethinyl group. In addition, studies have shown that the ethinyl estrogens act synergistically with the 19-nor progestins with respect to gonadotropin suppression. Whether a synergism of *non*-ethinyl estrogens with these progestins exists is uncertain. This amplification is the basis on which the contraceptive effectiveness of the very low–dose formulations rests, and the reason why the dosage in the original "sequential" regimens could not be lowered to currently desired levels.

Some of the adverse symptomatic effects of the estrogens are clearly dose related. Therefore it becomes important to have an in-depth understanding of their pharmacokinetics.

Mestranol

In vitro receptor studies[1] have demonstrated that mestranol is an inactive compound that becomes biologically active on conversion to ethinyl estradiol (EE). The degree of conversion varies with different species. Published human studies of the pharmacokinetics of EE derived from mestranol generally suffer from the small number of subjects used (usually <10) and from insufficient sampling frequency. Thus the general impression of a 50% conversion rate of mestranol to EE is based on rather uncertain grounds. In view of what is known today about the magnitude of interindividual and intraindividual variation with these compounds, a reinvestigation is clearly needed.

We[2] have recently examined the pharmacokinetic parameters derived from analysis of plasma EE levels after administration of a single dose of three bioequivalent norethindrone (1 mg)/EE (35 μg) formulations from different manufacturers, as well as three norethindrone (1 mg)/mestranol (50 μg) formulations from the same manufacturers. The protocol was designed as an open-label, three-way crossover study. Each subject received a two-tablet dose of a drug within the first 6 days of three consecutive menstrual cycles. This double dose was used to improve assay reliability. The drugs were assigned in a randomized sequence so that all three drugs were tested in each subject at a given estrogen dose. Twenty-four women took each of these EE-35 formulations; another 27 took the three mestranol-50 formulations. Some of the derived pharmacokinetic parameters are shown in Table I.

The data clearly show the delay in achieving maximum plasma EE levels derived from mestranol compared with EE as expected. Interestingly, the peak plasma concentration (C_{max}) values for EE derived from

From the Department of Obstetrics and Gynecology, Baylor College of Medicine.
Reprint requests: Joseph W. Goldzieher, MD, Department of Obstetrics and Gynecology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.
6/0/23775

Table I. Pharmacokinetic parameters for EE after oral administraion of single two-tablet doses of each oral contraceptive

		Formulation			
Variable	*Dose*	*Norcept*	*Ortho-Novum*	*Norinyl*	*Total*
AUC	1/35EE	1089 ± 570	993 ± 270	1024 ± 454	1036 ± 483*
(pg-hr/ml)	1/50ME	996 ± 454	940 ± 494	983 ± 429	963 ± 544†
C_{max}	1/35EE	171 ± 57	178 ± 45	174 ± 59	174 ± 67
(pg/ml)	1/50ME	175 ± 57	175 ± 69	175 ± 50	175 ± 72
T_{max}	1/35EE	1.3 ± 0.5	1.3 ± 0.6	1.4 ± 0.6	1.3 ± 0.5
(hr)	1/50ME	1.7 ± 0.7	1.8 ± 0.8	2.0 ± 0.8	1.9 ± 0.8
		$p = 0.008$	$p = 0.02$	$p = 0.003$	$p = < 0.0001$
K_{el}	1/35EE	0.08 ± 0.07	0.09 ± 0.07	0.11 ± 0.07	0.09 ± 0.09
	1/50ME	0.13 ± 0.10	0.18 ± 0.13	0.13 ± 0.10	0.15 ± 0.12
			$p = 0.04$		

Reproduced with permission from Brody JA, Turkes A, Goldzieher JW. Contraception 1989;40:269-84.

Values shown are mean ± SD for area under the plasma concentration/time curve ($AUC_{0\text{-}24}$) by the trapezoidal rule; peak plasma concentration (C_{max}); time to peak concentration (T_{max}); and the elimination rate constant (K_{el}).

*n = 24 × three trials.

†n = 27 × three trials.

Table II. Interindividual variability of EE plasma concentrations after administration of two 35 μg EE and two 50 μg mestranol pills

Parameter	*Mean ± SD*	*Range*	*CV (%)*
EE (35 μg EE pills)			
$AUC_{0/24}$ (pg-hr/ml)	1036 ± 483	284-2498	47
C_{max} (pg/ml)	174 ± 67	55-311	39
EE from mestranol (50 μg mestranol pills)			
$AUC_{0\text{-}24}$ (pg-hr/ml)	963 ± 544	215-2122	57
C_{max} (pg/ml)	175 ± 72	67-391	41

Data from Brody JA, Turkes A, Goldzieher JW. Contraception 1989;40:269-84.

CV, Coefficient variation.

Table III. Range of pharmacokinetic values for EE reported in the literature

$t_{1/2}$ (α)	0.5-2.4 hr
$t_{1/2}$ (β)	13.1-27.0 hr
$t_{1/2}$ (Ka)	0.2-0.4 hr
Bioavailability	0.38-0.48
K_{12}	0.182-0.249/hr
K_{21}	0.101-0.245/hr
K_{10}	0.193-0.309/hr
T_{max}	1-2 hr

Estimate of $t_{1/2}$ (α) has a wide range and needs further documentation.

Ranges of values indicated are derived from Newburger and Goldzieher.[6]

$t_{1/2}$ (α) is the half-life of drug disposition from the central to the peripheral compartment.

$t_{1/2}$ (β) is the elimination half-life.

$t_{1/2}$ (Ka) is the half-life of drug delivery to the central compartment, where Ka is the transfer rate constant in this direction.

K_{12} is the transfer rate constant from the central to the peripheral compartment; K_{21} is the transfer rate constant in the reverse direction.

K_{10} is the same as the elimination rate constant (K_{el}).

Bioavailability is calculated by dividing AUC after oral administration by AUC after intravenous administration with the same dose of drug.

T_{max} is the time of maximum plasma concentration of drug.

The theoretic model for these pharmacokinetic parameters is described in Goldzieher et al.[11]

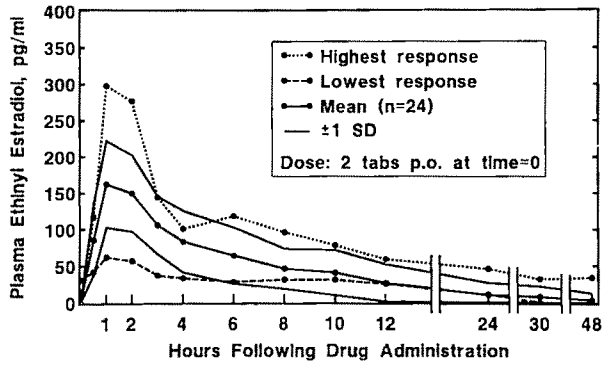


Fig. 1. Plasma EE contrations after administration of Norcept-E 1/35. A single dose of two tablets consisting of EE 35 μg/norethindrone 1 mg was given by mouth at time = 0. The mean ± SD is depicted along with highest and lowest individual responses. (Reproduced with permission from Brody SA, Turkes A, Goldzieher JW. Contraception 1989;40:269-84.)

50 μg of mestranol are identical with those derived from 35 μg of EE, both of which were given along with 1 mg of norethindrone. The mean area under the plasma concentration/time curve from 0 to 24 hours (AUC_{0-24}) values for EE derived from 50 μg of mestranol are clearly lower than those derived from 35 μg of EE as shown, but the very large intersubject variability keeps this difference from being statistically significant. From these data it may be concluded that EE from mestranol would yield about 70% of the C_{max} value of a similar dose of EE. Based on AUC_{0-24}, mestranol is somewhat less than 70% as bioavailable as EE. It appears that the usual estimate of 50% is probably low in terms of AUC.

The interindividual variation of plasma EE levels derived from EE or mestranol was similar: the coefficient of variation of AUC for EE was 47%; that for EE from mestranol was 57%. The coefficient of variation of C_{max} for EE was 39%; for EE derived from mestranol it was 41% (Table II).

These pharmacokinetic data are not necessarily concordant with pharmacodynamic observations. Studies of human endometrial response and measurements of hepatic synthesis of certain proteins suggest a potency of about 50% for mestranol compared with EE.[3-5] However, other studies of human endometrial response have found that the two compounds are bioequivalent over the range of 50 to 100 μg/day. Furthermore, studies of the effects on plasma gonadotropins, cortisol, testosterone, and androstenedione and their binding globulins and the effects on carbohydrate and lipid metabolism also found bioequivalence. These studies were conducted with estrogens alone rather than with commercial oral contraceptive formulations. It must be added, however, that these studies were not carried out on the numbers of patients we require in light of contemporary knowledge of interindividual variability.[6-10]

Table IV. Intraindividual variability of EE plasma concentration AUC_{0-24} after the administration of two 35 μg EE and 50 μg mestranol pills: three single-dose trials

	CV (%)	*Range**
EE (35 μg EE pills)	41	−66%-+71%
EE from mestranol (50 μg mestranol pills)	42	−70%-+92%

Data from Brody JA, Turkes A, Goldzieher JW. Contraception 1989;40:269-84.

CV, Coefficient of variation.

*The percent difference of high and low values from the mean of three trials per subject.

These findings are highly relevant to epidemiologic studies, which frequently analyze data sets in terms of the quantity of estrogen in contraceptive formulations without regard to the chemical nature of the estrogen. Thus some "high-dose" mestranol pills have a lower biologically active estrogen content than do some "low-dose" EE pills.[2]

EE

The literature on the pharmacokinetics of EE is substantial. There are large discrepancies in the values reported for the various parameters. These are probably because of limitations in sampling frequency, difficulties in measuring of plasma EE levels at clinically relevant dosages, and the effects of enterohepatic recirculation. The large interindividual variation seen with these drugs is a major confounding factor, accounting for the discrepant findings reported. Analysis of these difficulties has been presented elsewhere.[11] Our best estimate of the relevant pharmacokinetic parameters is shown in Table III. As Table III indicates, the half-lives for EE ($t_{1/2}\alpha$ and $t_{1/2}\beta$) have a very wide range. Oral bioavailability is relatively low, in the range of 38% to 48%. The transfer constants (K_{12}, K_{21}, and K_{10}) and the time to peak concentration are also quite variable. Our estimates of AUC_{0-24}, C_{max}, T_{max}, and K_{el} for a 50 μg dose from a large pharmacokinetic study are shown in Table II.

Large interindividual variation in the pharmacokinetics of EE is well recognized,[12] but has not been documented in studies with adequate sample size. The very large coefficients of variation of two parameters are presented in Table II. Fig. 1 illustrates the plasma levels for the mean, highest, and lowest responders in a group of 24 subjects given a 70 μg dose of EE. An analysis of time-integrated drug levels confirmed that the three commercial formulations tested were bioequivalent. Therefore the study design permitted an examination of intraindividual variation for single doses, an issue that has not been previously addressed (Table IV). For

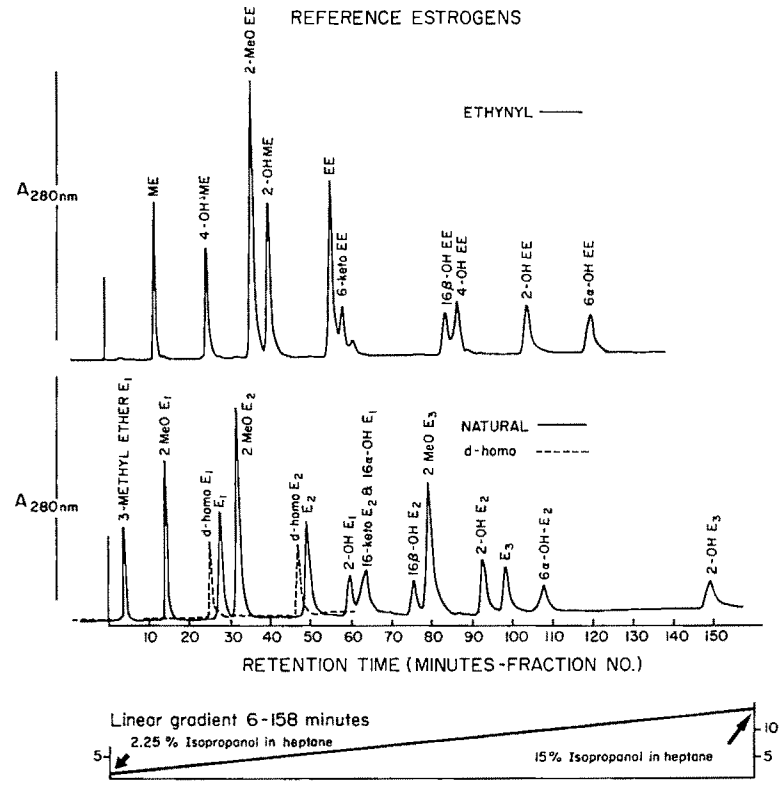


Fig. 2. Pattern of reference EE and non-ethinyl estrogens on a high-performance liquid chromatography. Chromegaprep Diol column, 9.6 mm by 50 cm, 10 μ; flow-7.5 ml/min; pressure, 1000 psi; linear gradient, 2.25 μ to 15% isopropanol in heptane in 158 minutes; detection by ultraviolet absorption at 280 nm. (Reproduced with permission from Butterworth-Heinemann. Williams MC, Goldzieher, JW. Steroids 1980;36:255-82.)

Table V. Representative plasma AUC_{0-24} values (pg-hr/ml) for EE and norethindrone after repeated trials in the same persons

Subject No.	*Ethynyl Estradiol*			*Norethindrone*		
	Norcept	*Ortho-Novum*	*Norinyl*	*Norcept*	*Ortho-Novum*	*Norinyl*
			1 + 35 EE formulations			
2	1002	431	855	29.2	23.1	49.3
9	252	499	569	32.2	55.7	46.8
12	1892	637	1390	79.7	77.2	91.7
13	1563	539	934	98.9	169.9	127.2
20	657	1594	2122	90.3	86.3	88.0
			1 + 50 ME formulations			
104	215	1230	658	30.3	73.7	120.5
106	1355	854	1080	175.0	33.9	15.6
3	768	963	696	109.8	86.6	31.6
16	1033	839	818	58.5	38.4	109.0
25	433	289	331	68.1	62.1	62.5

Reproduced with permission from Brody JA, Turkes A, Goldzieher JW. Contraception 1989;40:269-84.

both the 35 μg EE and the 50 μg mestranol preparations, there was a large intraindividual coefficient of variation: 41% to 42%. Within individual subjects the range of AUC varied enormously, from −70% to +92% of the mean of the three trials. Typical examples of this variability are shown in Table V. The unexpectedly large intraindividual variance, which showed no product-specific tendency, is apparent from the table. Moreover, the rank order of values for AUC_{EE} in the three trials per individual does not correlate with the rank order of the three results for AUC_{NET} (norethindrone), a point that has already been made by Fotherby.[13]

A prevailing opinion has been that the conjugates of

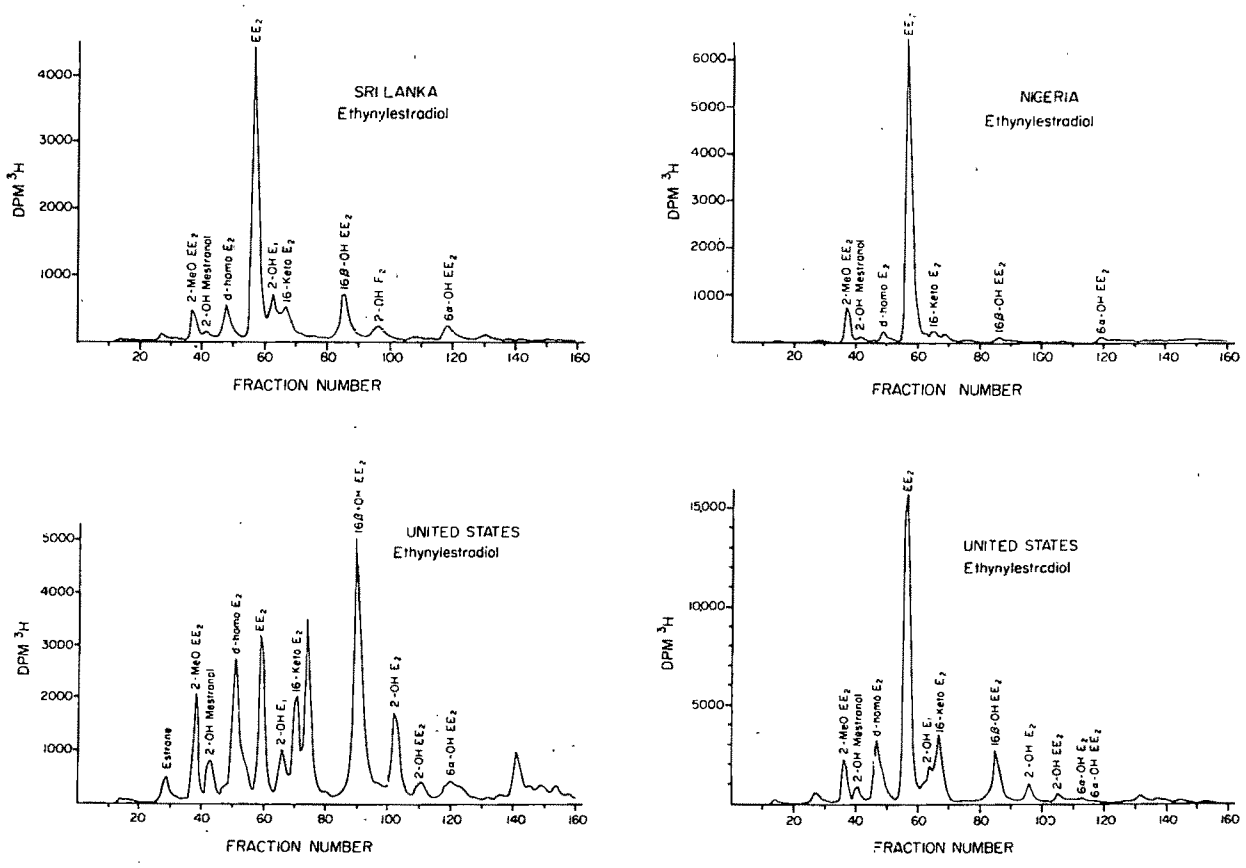


Fig. 3. Representative patterns of urinary mestranol and EE metabolites in women from Sri Lanka, Nigeria and the United States. High-performance liquid chromatography Chromegaprep Diol column, 9.6 mm by 50 cm; 10 μ; flow-7.5 ml/min; pressure, 1000 psi; linear gradient, 2.25% to 15% isopropanol in heptane in 158 minutes, scintillation counting of 1-minute fractions. Results from the two U.S. women illustrate the wide range of oxidative metabolism seen in this population. (Reproduced with permission from Butterworth-Heinemann. Williams MC, Goldzieher JW. Steroids 1980;36:255-82.)

EE, specifically the 3-sulfates, have relatively long half-lives and through enterohepatic recirculation might be deconjugated to provide a slow-release reservoir of EE. We have synthesized the various sulfates and have carried out clinical trials with these substances. A detailed analysis of the pharmacokinetic parameters has been published.[14] In summary, only 3.4% of intravenously administered and 11.4% of orally administered 17-sulfate appeared in the blood as free EE; with the 3-sulfate the conversion rates were 13.7% and 20.7%, respectively, which suggests that the sulfates are not important reservoirs. Moreover, the half-lives of free and sulfoconjugated EE are similar, ranging from 8.8 to 11.2 hours. Finally, the $t_{1/2}$ of the 17-sulfates after intravenous administration was similar to that of free EE. As expected, much more individual variation was encountered with oral administration than with the intravenous route. This phenomenon was apparent for both the 3- and 17-sulfates, as reflected in measurement of the total circulating EE sulfates.

Ethnic differences

Several investigators[15-17] have studied the pharmacokinetics of contraceptive steroids in various populations. Substantial differences in bioavailability have been noted. In comtemporary perspectives, the sample sizes studies and the blood sampling schedules used raise questions about the impact of interindividual variability as a possible confounding factor in the interpretation of these data. Although evidence of quantitative differences between populations is perhaps now less certain than was formerly thought, qualitative studies unequivocally support the conclusion that these population differences are real. In published studies[18] we have analyzed the pattern of urinary conjugates (glucuronides and sulfates) of EE after oral administration of radioactively labeled material to women in the United States, Sri Lanka, and Nigeria. In the three populations studied, the proportions of glucuronides and sulfoconjugates were similar: about 70% and 18%, respectively. However, the patterns of glucuronide con-

jugates seemed to differ. Further evidence of metabolic differences between populations can be demonstrated by examination of the oxidative metabolites of EE itself.

High-performance liquid chromatography profiling permits high-resolution identification of the various metabolites of EE (Fig. 2). There was a consistent difference, with Nigerian women (n = 10) displaying the least, Sri Lanka women (n = 10) intermediate, and American women (n = 6) the highest degree of oxidative metabolism, as evidenced by multiple chromatographic peaks identifying distinct metabolic products (Fig. 3). Such findings rule out the possibility that the observed pharmacokinetic differences between ethnic groups were from the confounding effects of interindividual variation. More important, they raise interesting questions regarding ethnic differences in the enterohepatic metabolism of EE. Further studies will be needed to address the role of nutritional factors, intestinal bacterial flora, genetic steroid-metabolic differences, and other as yet undiscovered influences on the metabolism of these compounds.

REFERENCES

1. Kappus H, Bolt HM, Remmer H. Affinity of ethynylestradiol and mestranol for the uterine estrogen receptor and for the microsomal mixed function oxidase of the liver. J Steroid Biochem 1973;4:121-8.
2. Brody SA, Turkes A, Goldzieher JW. Pharmacokinetics of three bioequivalent norethindrone/mestranol-50μg and three norethindrone/ethynyl estradiol-35μg OC formulations: are "low-dose" pills really lower? Contraception 1989;40:269-84.
3. Delforge JP, Ferin J. A histometric study of two estrogens: ethinylestradiol and its 3-methyl-ester derivative (mestranol); their comparative effect upon the growth of the human endometrium. Contraception 1970;1:57-63.
4. Teter J, Stupnicki R. A comparative study of the estrogenic potential of two synthetic estrogens (mestranol and ethinylestradiol). Acta Cytol 1971;15:167-70.
5. Goldzieher JW, Maqueo M, Chenault CB, Woutersz TB. Comparative studies of the ethynyl estrogens used in oral contraceptives. I. Endometrial response. Am J Obstet Gynecol 1975;122:615-8.
6. Goldzieher JW, de la Pena A, Chenault CB, Cervantes A. Comparative studies of the ethynyl estrogens used in oral contraceptives. III. Effect on plasma gonadotropins. Am J Obstet Gynecol 1975;122:625-36.
7. Goldzieher JW, Chenault CB, de la Pena A, Dozier TS, Kraemer DC. Comparative studies of the ethynyl estrogens used in oral contraceptives: effects with and without progestational agents on plasma cortisol and cortisol binding in humans, baboons, and beagles. Fertil Steril 1977;28:1182-90.
8. Goldzieher JW, Chenault CB, de la Pena A, Dozier TS, Kraemer DC. Comparative studies of the ethynyl estrogens used in oral contraceptives: effects with and without progestational agents on plasma androstenedione, testosterone, and testosterone binding in humans, baboons, and beagles. Fertil Steril 1978;29:388-96.
9. Goldzieher JW, Chenault CB, de la pena A, Dozier TS, Kraemer DC. Comparative studies of the ethynyl estrogens used in oral contraceptives. VI. Effects with and without progestational agents on carbohydrate metabolism in humans, baboons, and beagles. Fertil Steril 1978;30:146-53.
10. Goldzieher JW, Chenault CB, de la Pena A, Dozier TS, Kraemer DC. Comparative studies of ethynyl estrogens used in oral contraceptives. VII. Effects with and without progestational agents on ultracentrifugally fractionated plasma lipoproteins in humans, baboons, and beagles. Fertil Steril 1978;30:522-33.
11. Newburger J, Goldzieher JW. Pharmacokinetics of ethynyl estradiol: a current view. Contraception 1985;32:33-44.
12. Back DJ, Breckenridge AM, Crawford FE, Maciver M, Orme ML'E, Rowe PH. Interindividual variation and drug interactions with hormonal steroid contraceptives. Drugs 1981;21:46-61.
13. Fotherby K. Pharmacokinetics of ethynyloestradiol in humans. Methods Find Exp Clin Pharmacol 1982;4:131-41.
14. Goldzieher JW, Mileikowsky G, Newburger J, Dorantes A, Stavchansky SA. Human pharmacokinetics of ethynyl estradiol 3-sulfate and 17-sulfate. Steroids 1988;51:63-79.
15. Goldzieher JW, Dozier TS, de la Pena A. Plasma levels and pharmacokinetics of ethynyl estrogens in various populations. I. Ethynylestradiol. Contraception 1980;21:1-16.
16. Fotherby K, Akpoviroro J, Abdel-Rahman HA, et al. Pharmacokinetics of ethynyloestradiol in women from different populations. Contraception 1981;23:487-96.
17. Fotherby K. Variability of pharmacokinetic parameters for contraceptive steroids. J Steroid Biochem 1983; 19:817-20.
18. Williams MC, Goldzieher JW. Chromatographic patterns of urinary ethynyl estrogen metabolites in various populations. Steroids 1980;36:255-82.

Pharmacokinetics of the contraceptive steroids levonorgestrel and gestodene after single and multiple oral administration to women

Wilhelm Kuhnz, PhD
Berlin, West Germany

Little is known about the pharmacokinetics of the two progestins levonorgestrel and gestodene during long-term administration compared with single-dose pharmacokinetics. The predictive value of single-dose administration for the pharmacokinetic behavior of a progestin during long-term treatment was investigated for two triphasic oral contraceptives. One contained levonorgestrel and the other gestodene, each in combination with ethinyl estradiol. In eight Japanese women who received the levonorgestrel-containing formulation over a treatment cycle, steady-state trough levels of levonorgestrel were higher than those obtained by computer simulation based on single-dose administration. An analogous observation was made in a group of 10 white women who received the gestodene-containing formulation. A close correlation between gestodene and sex hormone–binding globulin concentrations was demonstrated for eight subjects; the other two patients already had initially high sex hormone–binding globulin levels. Ethinyl estradiol–induced production of sex hormone–binding globulin seems to be a major factor that contributes to the accumulation of the two progestins in the plasma. Computer simulation, based on single-dose pharmacokinetics, allows an estimation of this contribution. (AM J OBSTET GYNECOL 1990;163:2120-7.)

Key words: Pharmacokinetics, synthetic progestins, long-term administration, steady-state concentrations, sex hormone–binding globulin

Levonorgestrel (LNG) and gestodene (GEST) are two synthetic progestins that are used in combination with ethinyl estradiol (EE_2) as oral contraceptives. Whereas LNG has been used for many years, GEST was introduced only a few years ago, and accordingly, a fairly large number of clinical pharmacokinetic studies have been performed with LNG-containing oral contraceptives, whereas a comparatively smaller number of analogous studies have been reported for GEST. Consequently, the pharmacokinetic characteristics of LNG after single-dose administration (intravenous and oral) are well known,[1] whereas analogous information on the pharmacokinetics of GEST has become available more recently.[2, 3] Compared with single-dose pharmacokinetics, much less published data are available on the administration of both progestins throughout a whole treatment cycle in healthy women.[3, 4, 9]

Because single-dose pharmacokinetics of a particular progestin hardly reflects the real situation of women taking an oral contraceptive for long durations, usually for many years, at least one important question must be addressed: Is the pharmacokinetic profile of a particular progestin the same after single- and multiple-dose administration or, in others words, is it possible to simply predict the pharmacokinetic behavior of a progestin during long-term treatment from the characteristics obtained after the administration of a single dose?

In this article this issue will be discussed for two triphasic oral contraceptives, one containing LNG and the other GEST, in combination with EE_2 as the estrogenic component.

Material and methods

Study design: Study I. This study was made up of two parts. The first included a group of seven healthy Japanese women between 22 and 34 years old, who received a single-coated tablet of each of the three dosage forms of the triphasic formulation (Triquilar) at the adequate time of the cycle (days 1, 7, and 13).

Blood samples were taken at the following times: before administration and 0.5, 1, 2, 3, 4, 8, and 24 hours after drug intake.

The second part of the study included eight healthy Japanese women between 21 and 35 years, who received the triphasic formulation during a whole cycle according to the following schedule: days 1 through 6: 0.05 mg LNG + 0.03 mg EE_2; days 7 through 11: 0.075 mg LNG + 0.04 mg EE_2; and days 12 through 21: 0.125 mg LNG − 0.03 mg EE_2.

Blood samples were taken during this period at the

From the Research Laboratories, Schering AG.
Reprint requests: Wilhelm Kuhnz, PhD, Department of Pharmacokinetics, Schering AG, Müllerstraße 170-178, D-1000 Berlin 65, West Germany.
6/0/23263

Table I. Pharmacokinetic parameters (mean values) of LNG obtained from seven Japanese women who received three single-coated tablets of the triphasic oral contraceptive at adequate times during one cycle; parameters were calculated on the basis of an open two-compartment model; concentration values of LNG obtained after single administration of 0.05 mg LNG + 0.03 mg EE_2 were not suitable for model-fitting and therefore were not included

Parameter	*Dose of LNG (mg)*	
	0.075	*0.125*
C_{max} (ng/ml)	1.6	2.6
t_{max} (hr)	1.0	1.2
AUC (0-24 hr) (ng · ml^{-1} · hr)	8.5	17.1
AUC (ng · ml^{-1} · hr)	11.2	20.0
$t^{*}_{1/2}$ (hr)	13.1	13.1
V_{SS} (l)	90.6	85.0
Cl (ml · min^{-1} · kg^{-1})	2.0	1.9

V_{SS}, Apparent volume of distribution at steady state; *Cl*, clearance.

*Terminal half-life calculation was based on two concentration values only.

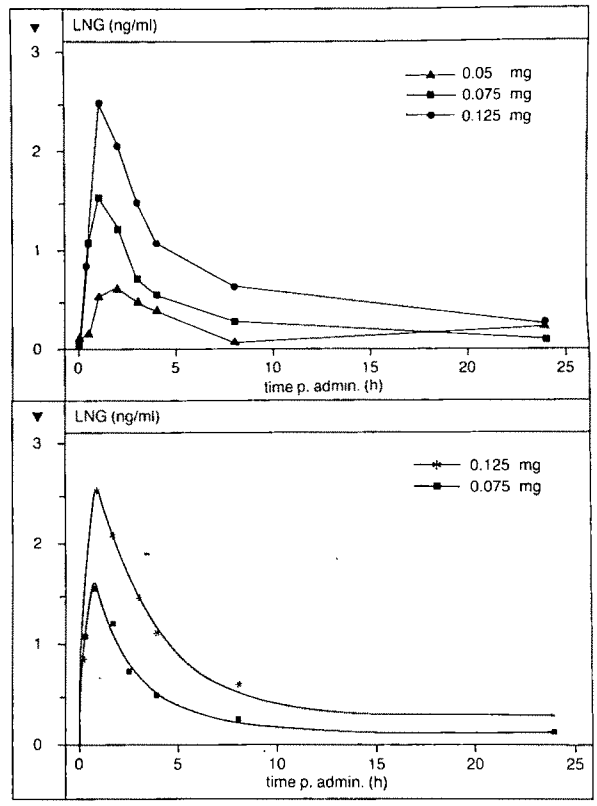


Fig. 1. Mean concentrations of LNG in plasma of seven Japanese women who received single doses of 0.05, 0.075, and 0.125 mg of LNG as coated tablets of a triphasic oral contraceptive at adequate times (days 1, 7, and 13) of the cycle. Directly obtained LNG concentration/time curves *(top)* are presented along with curves fitted on the basis of an open two-compartment model *(bottom)*.

following times: days 2, 4, 6, 8, 10, 12, 16, 18, and 20 before drug administration, and day 21 before drug administration and 0.5, 1, 2, 4, 8, 24, and 48 hours after drug intake.

All blood samples were collected in heparinized tubes and centrifuged, and plasma samples were frozen at −20° C until analysis.

Study design: Study II. This study was designed as an open investigation with 10 healthy white women between 23 and 39 years, who received a single-coated tablet of the triphasic preparation (Milvane) that contained 0.1 mg of GEST together with 0.03 mg of EE_2 on day 21 of a pretreatment cycle.

Blood samples were taken at the following times: before administration and 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 48, and 72 hours after drug intake.

After a wash-out phase of 7 days, the same women received, in the second part of the study, the triphasic formulation during a whole cycle: days 1 through 6: 0.05 mg GEST + 0.03 mg EE_2; days 7 through 11: 0.07 mg GEST + 0.04 mg EE_2; and days 12 through 21: 0.1 mg GEST + 0.03 mg EE_2.

Blood samples were taken at the following times: days 2 and 7: before and 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours after drug administration, and day 21: before and 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours after drug intake.

Additional blood samples were obtained on days 3, 5, 8, 10, 12, 14, 16, 18, and 20 before drug administration. All blood samples were kept at 4° C until coagulation; the serum was separated and stored at −20° C until analysis.

Analytic determinations. LNG was determined in all plasma samples of study I by a specific radioimmunoassay.[5] The serum samples of study II were analyzed for total concentration of GEST by a specific radioimmunoassay.[6] The free fraction of GEST, as well as the distribution of GEST over the serum-binding proteins albumin and sex hormone–binding globulin (SHBG), was determined by ultrafiltration[7] with and without heat treatment of the serum samples.[8]

In study II SHBG and cortisol-binding globulin (CBG) concentrations were measured by radioimmunologic means with two commercially available assay kits (SHBG: Diagnostic Products Corp., Los Angeles, Calif.; CBG: IRE Medgenix, Fleurus, Belgium).

Pharmacokinetic evaluation. Plasma concentrations of LNG, which were obtained during study I, were used for the model-free evaluation of maximum drug-concentration (c_{max}), time of maximum concentration (t_{max}), and area under the curve (AUC). In addition, the LNG

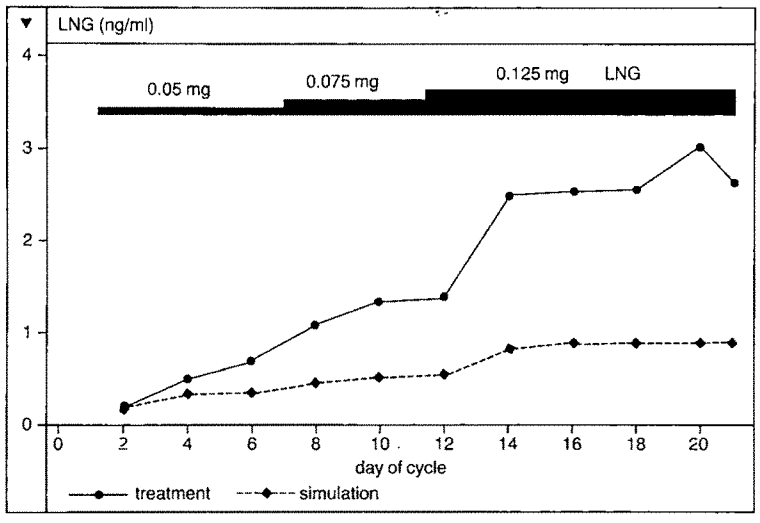


Fig. 2. Mean trough concentrations of LNG in plasma of eight Japanese women who received the triphasic oral contraceptive during one cycle. Experimentally measured and computer-simulated values are presented.

concentrations in plasma obtained after single-dose administration were evaluated by model-fitting (TOPFIT, Thomae GmbH, Biberach, West Germany) on the basis of an open two-compartment model. The serum concentrations of GEST obtained in study II were evaluated by model-fitting only. Apparent oral clearances and volumes of distribution were calculated for both progestins assuming 100% bioavailability.[2, 5, 10] The data obtained by model-fitting were used for the simulation of progestin concentrations in plasma (serum) during treatment with the respective triphasic formulations over a whole treatment cycle.

Accumulation factors were calculated as follows:

$$R = \frac{1}{1 - e^{\left(-\frac{\ln 2}{t_{1/2}} \cdot \tau\right)}}$$

$$R^* = \frac{C_{ss}^{min}\ (TC)}{C_{ss}^{min}\ (SC)}$$

where $t_{1/2}$ = terminal half-life; τ = dosing interval; C_{ss}^{min} = minimum concentration at steady state; TC = treatment cycle; and SC = simulated treatment cycle.

$$R^{**} = \frac{AUC\ (0\text{-}24\ hr)\ TC}{AUC_{SD}}$$

where SD = single dose.

Results

Study I: Pharmacokinetics of LNG after single-dose administration to Japanese women. The maximum concentration values of LNG, which were observed in the plasma of the seven women after single oral administration of the three different dosages (0.05, 0.075, and 0.125 mg of LNG), were 0.72 ± 0.34 ng/ml, 1.83 ± 0.61 ng/ml, and 2.74 ± 0.96 ng/ml, respectively. The corresponding t_{max} values were 2 ± 1 hour, 1.1 ± 0.7 hour, and 1.1 ± 0.6 hour, respectively. The observed AUC (0 to 24 hours) − values were 5.0 ± 4.1 ng * ml^{-1} * hr, 8.8 ± 5.3 ng * ml^{-1} * hr, and 17.1 ± 6.4 ng * ml^{-1} * hr, respectively. The time course of LNG levels in the plasma is shown in Fig. 1.

The mean LNG concentration values in plasma obtained after the administration of a single dose of 0.075 and 0.125 mg of LNG, respectively, were fitted on the basis of an open two-compartment model (Fig. 1). The derived pharmacokinetic parameters were identical to those determined without model-fitting (Table I).

A computer simulation of LNG trough levels in plasma during a 21-day treatment period was performed on the basis of those pharmacokinetic parameters that were obtained with high-dose LNG (0.125 mg) except that the terminal half-life was set to a value of 22 hours, which represented the experimentally observed value at the end of one treatment cycle in the second part of study I.

The result of this simulation is presented in Fig. 2, along with the experimentally obtained concentration values. The comparison of both curves demonstrated the following: (1) that simulated trough levels of LNG increased during treatment, reaching steady state on day 16 of treatment, and (2) that experimentally measured LNG trough levels closely followed simulated ones; however, they increased more markedly and reached a considerably higher plateau during the last week of treatment. Both curves demonstrate an increase in LNG plasma levels during 1 month of treatment.

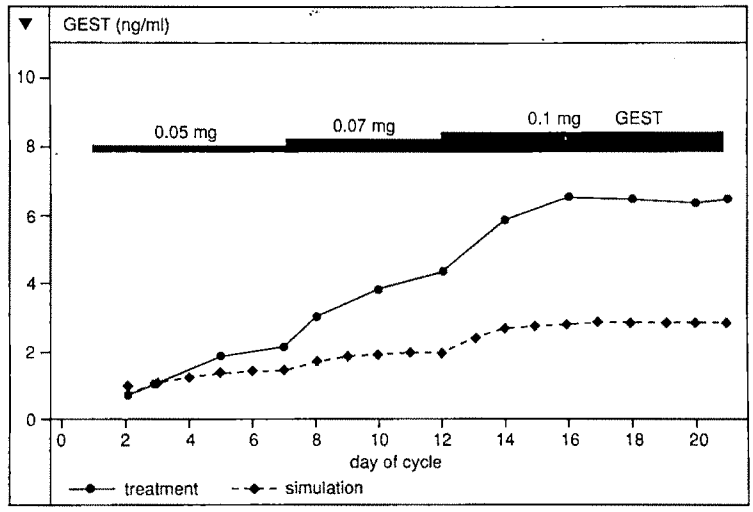


Fig. 3. Mean trough concentrations of GEST in the serum of 10 white women who received a GEST-containing triphasic oral contraceptive during one cycle. Experimentally measured and computer-simulated values are presented.

Table II. Pharmacokinetic parameters of LNG (mean ± SD) of eight Japanese women who received the triphasic formulation during one treatment cycle; data were calculated without model-fitting

Parameter	*Day 21 of treatment*
C_{max} (ng/ml)	6.2 ± 2.1
t_{max} (hr)	1.3 ± 0.5
AUC (0-24 hr) (ng · ml^{-1} · hr)	80.7 ± 34.8
AUC (ng · ml^{-1} · hr)	154.2 ± 69.7
$t_{1/2}$ (hr)	22.1 ± 5.7

Table III. Pharmacokinetic parameters of GEST (mean ± SD) obtained by model-fitting based on total and free concentrations in serum after single oral administration of a coated tablet containing 0.1 mg of GEST together with 0.03 mg of EE_2 on day 21 of a pretreatment cycle to 10 women

Parameter	*Total GEST*	*Free GEST*
C_{max} (ng/ml)	7.2 ± 3.2	0.09 ± 0.04
t_{max} (hr)	1.3 ± 1.1	1.3 ± 1.1
AUC (0-24 hr) (ng · ml^{-1} · hr)	50.6 ± 22.9	0.65 ± 0.25
AUC (ng · ml^{-1} · hr)	74.3 ± 37.5	0.97 ± 0.46
$t_{1/2}$ (hr)	15.8 ± 4.9	15.9 ± 5.4
V_{ss} (l)	32.2 ± 19.4	2242 ± 974
Cl (ml · min^{-1} · kg^{-1})	0.48 ± 0.25	34.7 ± 15.8

V_{ss}, Apparent volume of distribution at steady state; *Cl*, clearance.

An accumulation factor (R) of 1.9 can be calculated for LNG from a terminal half-life of 22 hours and a dosing interval of 24 hours. However, this only applies if accumulation is determined only by the terminal half-life of the drug and the dose interval chosen. If this was the case, the ratio (R*) of experimentally determined and simulated steady-state trough levels should be unity. Likewise, the ratio (R**) of AUC obtained on day 21 of the pretreatment cycle and AUC (0 to 24 hours) obtained on day 21 of the treatment cycle should also be unity. However, a value of 2.9 was calculated for the former and a value of 4.0 for the latter ratio.

Study I: Pharmacokinetics of LNG after multiple dose administration to Japanese women. Mean trough concentrations of LNG, which were measured during the treatment cycle, reached a steady state on about day 16 (Fig. 2).

On the last day of treatment (day 21), maximum concentrations of LNG in the plasma were observed 1.3 ± 0.5 hour after administration and amounted to 6.2 ± 2.1 ng/ml.

Postmaximum drug concentrations in plasma declined, with a mean half-life of disposition of 22.1 ± 5.7 hours. The AUC (0 to 24 hours) was 80.7 ± 34.8 ng * ml^{-1} * hr (Table II).

Study II: Pharmacokinetics of GEST after single-dose administration to white women. The maximum concentration values of GEST, which were observed in the serum of the 10 women after a single oral administration of 0.1 mg of GEST together with 0.03 mg of EE_2, were observed after 1.3 ± 1.1 hour and amounted to 7.2 ± 3.2 ng/ml. The terminal half-life of disposition was 15.8 ± 4.9 hours. The AUC was 74.3 ± 37.5 ng * hr * ml^{-1}. Total serum clearance was calculated to 0.48 ± 0.25 ml * min^{-1} * kg^{-1} (Table III). Protein binding of GEST and drug distribution over the binding proteins were determined before drug administration and 1 and 10 hours after drug intake, and they were identical in all cases. The free fraction of GEST

Table IV. Protein binding of GEST and concentration of SHBG and CBG (mean ± SD) in the serum of 10 women on day 21 of a pretreatment cycle and on day 21 of treatment

Parameter	*Day 21 pretreatment cycle*	*Day 21 treatment cycle*
GEST		
Free fraction (%)	1.37 ± 0.31	0.81 ± 0.15
SHBG-bound (%)	66.5 ± 6.9	76.6 ± 5.8
Albumin-bound (%)	32.2 ± 6.6	22.6 ± 5.8
SHBG (nmol/L)	90.3 ± 36.0	198.8 ± 52.0
CBG (μg/ml)	37.4 ± 5.2	84.9 ± 11.4

Table V. Pharmacokinetic parameters of GEST (mean ± SD) obtained by model-fitting based on total and free concentrations in serum of 10 women (on day 21 of treatment) who received the triphasic preparation during one cycle

Parameter	*Total GEST*	*Free GEST*
C_{max} (ng/ml)	18.2 ± 4.5	0.15 ± 0.04
t_{max} (hr)	0.8 ± 0.4	0.9 ± 0.4
AUC (0-24 hr) (ng · ml^{-1} · hr)	226.6 ± 77.1	1.8 ± 0.6
AUC (ng · ml^{-1} · hr)	422.6 ± 178.5	3.5 ± 1.5
$t_{1/2}$ (hr)	22.0 ± 4.1	22.9 ± 5.4

was determined to 1.37% ± 0.31% and the fraction bound to SHBG and albumin was 66.5% ± 6.9% and 32.2% ± 6.6%, respectively. CBG concentrations in the serum were 37.4 ± 5.2 μg/ml; SHBG concentrations were 90.3 ± 36.0 nmol/L (Table IV). The free concentrations of GEST were used to calculate the pharmacokinetic parameters of free GEST by model-fitting (Table III).

A computer simulation of GEST trough levels in the serum during a complete treatment cycle of 21 days was performed on the basis of the data obtained after single-dose administration on day 21 of the pretreatment cycle (Fig. 3) except that the terminal half-life was set to a value of 22 hours, which represented the experimentally observed value at the end of one treatment cycle in the second part of study II. Both curves showed similar time course except that the experimentally determined trough levels of GEST, which reached a steady state on approximately day 16, increased more markedly and reached a considerably higher plateau in the last week of treatment compared with the simulated trough levels. An accumulation factor (R) of 1.9 can be calculated from a terminal half-life of 22 hours and a dosing interval of 24 hours. Values calculated for R* and R** were 2.3 and 3.4, respectively. Corresponding values of R* and R** for the free GEST were 1.3 and 1.9, respectively.

Study II: Pharmacokinetics of GEST after multiple-dose administration to white women. Mean trough concentrations of GEST increased during the treatment cycle and reached a steady state on about day 16 (Fig. 3). On the last day of treatment (day 21), maximum concentrations of GEST in the serum were observed 0.8 ± 0.4 hour after administration and amounted to 18.2 ± 4.5 ng/ml. The terminal half-life of disposition was 22.0 ± 4.1 hours. The AUC (0 to 24 hours) was 226.6 ± 77.1 ng * hr * ml^{-1}; corresponding AUC value was 422.6 ± 178.5 ng * hr * ml^{-1}.

The corresponding pharmacokinetic parameters calculated from the free GEST concentrations in serum are also presented (Table V).

The free fraction of GEST was determined in the serum samples obtained throughout the treatment cycle. During treatment free GEST fractions decreased from a pretreatment value of 1.37% ± 0.31% to a value of 0.81% ± 0.15% on day 21 of treatment. The fractions of GEST bound to SHBG and albumin were 76.6% ± 5.8% and 22.6% ± 5.8%. The mean SHBG and CBG levels on day 21 were 198.8 ± 52.0 nmol/L and 84.9 ± 11.4 μg/ml, respectively (Table IV).

SHBG concentrations showed a steady increase in 8 of 10 women throughout treatment and were correlated significantly with the corresponding GEST concentrations in serum (Fig. 4). In two women relatively high SHBG concentrations were already observed when treatment started, and they remained nearly unchanged throughout treatment. The correlation coefficients for total and free GEST concentrations, respectively, and the corresponding SHBG levels were calculated individually, and for eight women were in the range of 0.7101 and 0.9112 (mean, 0.8195) and 0.5553 and 0.9126 (mean, 0.7914), respectively. In two women there was no correlation between both parameters.

Comment

Two different triphasic oral contraceptives were investigated, one containing LNG and the other GEST as progestagenic compound. However, the studies were carried out in two different ethnic populations and with slightly different design, since they were performed independent of each other and not particularly for comparative purposes. Nevertheless, it became obvious that both progestins, when administered in combination with EE_2 as triphasic formulations, showed very similar concentration profiles during a treatment cycle.

Pharmacokinetic parameters of LNG obtained after the administration of a single oral dose to Japanese women were in good accordance with published data on single dose administration in white subjects,[1] although somewhat higher drug levels were found in the plasma of Japanese women because of their comparatively lower body weight. Because terminal half-life

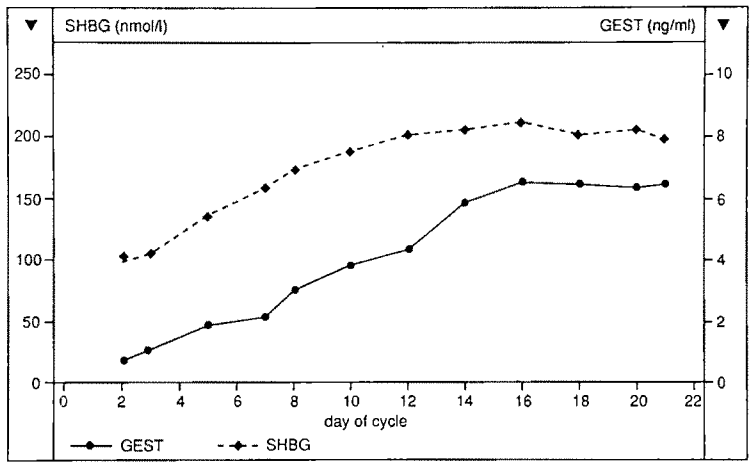


Fig. 4. Mean serum levels of SHBG and GEST in 10 white women during one treatment cycle.

estimation was based on 8 and 24 hour values only, the half-life of LNG was somewhat shorter than reported previously.[5] The pharmacokinetic parameters of LNG obtained with and without the use of model-fitting were identical, thus proving the validity of the model. During 1 month of treatment with the triphasic preparation, plasma trough levels of LNG increased concomitantly with the increasing dose of LNG administered and reached a steady state on about day 16 of treatment. Simulated trough levels of LNG showed a similar time course, although the rise was less steep and the steady state reached was considerably lower compared with experimentally determined values. Computer simulation assumed linear pharmacokinetics of LNG during long-term administration and any deviation of experimentally determined trough levels from the simulated ones indicated additional influences on the drug's disposition, which were due to long-term treatment and did not occur during single-dose administration. At least two different factors, which may affect the drug's distribution and disposition from plasma, have been reported for contraceptive steroids; one is an impairment of hepatic metabolic capacity, whereas the other relates to changes in protein binding. There have been numerous reports on impairment of hepatic drug-metabolizing enzymes in women under oral contraceptive therapy.[11-13]

This was true for formulations containing LNG and other progestins, and although GEST in particular was not yet investigated, it can be assumed that it also follows the general line. For steroids such as LNG and GEST, which are characterized by a restrictive hepatic elimination, any impairment of the metabolic capacity during long-term treatment could lead to higher drug levels in the plasma than anticipated from single-dose pharmacokinetics. On the other hand, it is well known that concomitant administration of EE_2 can increase SHBG concentration throughout treatment, and because LNG and GEST bind with a high affinity to SHBG, an increase in total drug levels in the serum is the result. Although SHBG was not determined in the present study with Triquilar, most published information on this particular triphasic formulation indicates about a 100% increase in SHBG levels during a treatment cycle,[14-16] although a smaller increase has been reported as well.[17]

Because computer simulation did not account for any changes in hepatic metabolic capacity or in plasma protein binding during treatment, a comparison of simulated and experimentally obtained trough level curves allowed an estimation of the relative contribution of both factors to the observed increase in LNG concentrations in plasma. Therefore a ratio of 2.9 for the two steady-state concentrations indicated about threefold higher LNG concentrations during treatment than anticipated on the basis of single-dose pharmacokinetics alone.

Similarly, when the ratio of the AUC obtained after single-dose administration and the AUC of one dosing interval on day 21 of the treatment cycle was examined, fourfold higher drug levels were found during treatment. Thus the relative contribution of factors other than the relationship of terminal half-life and dosing interval to the total accumulation of LNG in the plasma was about 75%.

A very similar observation was made with GEST. Data obtained from single-dose administration to white women were in good agreement with those observed in previous studies (Kuhnz W, et al. Unpublished data). However, compared with a previously performed study on single-dose administration of a GEST-containing formulation, a slightly different protein binding pattern of GEST was observed in the present study. Compared with the former study, in the present study, higher SHBG concentrations and a higher proportion of GEST bound to SHBG were found in the serum.

and a smaller fraction of GEST unbound was observed.

The most likely explanation for this phenomenon is that only women who had not previously taken any oral contraceptives were included in the previously performed study, whereas in the present study most women were regularly taking oral contraceptives and only stopped taking the pill between 2 and 6 months before the study started. The administration of GEST over a whole treatment cycle resulted in increasing trough levels, which reflected the triphasic pattern of the GEST doses administered. The increasing trough levels were paralleled by a 2.2-fold increase in SHBG concentrations in serum, and a close correlation between both parameters was found in 8 of 10 women. However, two women showed no such correlation; they already had high pretreatment levels of SHBG that decreased slightly during treatment, although GEST concentrations increased in a similar way as in the other women. A very similar observation has been reported previously on LNG,[18] in which seven of nine subjects had a close positive correlation between SHBG and LNG levels, whereas two other women had no such correlation. Both women already started with high SHBG levels, which decreased during treatment. In the present study a comparison with experimentally observed steady-state concentrations revealed 2.3-fold higher GEST concentrations during treatment than those calculated on the basis of single dose pharmacokinetics.

Likewise, an evaluation of the AUCs revealed 3.4-fold higher drug levels during treatment. Therefore the relative contribution of serum protein binding and hepatic metabolic impairment to the total concentration of GEST in serum at steady state was about 71%.

When free GEST concentrations were considered in the same way, only 1.3-fold and 1.9-fold higher drug levels were calculated. Although this was still somewhat higher than unity, it clearly demonstrated the influence of protein binding for the observed increase in GEST trough levels in serum. For restrictively cleared drugs such as LNG and GEST, an increase in serum protein binding affects not only volume of distribution and thus drug levels in serum, but also the progestin's terminal half-life. Because of the relative increase in the SHBG-bound fraction of the progestin, which is not readily metabolized by the liver, the drug's terminal half-life will be prolonged, which in fact was observed with GEST.

Both increasing progestin levels and prolonged terminal half-lives could also be explained by an impairment of hepatic metabolism. However, where there is evidence for the contribution of protein binding, it remains unclear whether metabolic impairment plays an important role.

In conclusion, it became apparent that the pharmacokinetic parameters obtained after single-dose administration of either of the two progestins cannot be used to predict their pharmacokinetic behavior completely during a treatment cycle; the main reason is the continuously altered protein binding of the progestins because of EE_2-induced SHBG synthesis.

However, single-dose pharmacokinetics of a progestin, together with computer simulation based on pharmacokinetic modeling, can be a useful tool to detect any deviations from linear pharmacokinetics during repeated administration. Along with additional information, for example, on altered binding capacity of SHBG, the interpretation of experimentally obtained results is greatly facilitated.

REFERENCES

1. Orme ML'E, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1983;8:95-136.
2. Täuber U, Tack JW, Matthes H. Single dose pharmacokinetics of gestodene in women after intravenous and oral administration. Contraception 1989;40:461-79.
3. Düsterberg B, Tack JW, Krause W, Hümpel M. Pharmacokinetics and biotransformation of gestodene in man. In: Elstein M, ed. Gestodene. Carnforth, England: Parthenon Publishing, 1987:35-44.
4. Kuhl H, Jung-Hoffmann C, Heidt F. Alterations in the serum levels of gestodene and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 75 μg gestodene. Contraception 1988;38:477-86.
5. Hümpel M, Wendt H, Pommerenke G, Weiss C, Speck W. Investigations of pharmacokinetics of levonorgestrel to specific consideration of a possible first pass effect in women. Contraception 1978;17:207-20.
6. Nieuweboer B, Tack J, Täuber U, Hümpel M, Wendt H. Development and application of a radioimmunoassay of the new progestogen gestodene. Contraception 1989;40:313-23.
7. Kuhnz W, Pfeffer M, Al-Yacoub G. Protein binding of the contraceptive steroids gestodene, 3-keto-desogestrel and ethinylestradiol in human serum. J Steroid Biochem 1990;35:313-8.
8. Hammond GL, Lähteenmäki PLA, Lähteenmäki P, Luukkainen T. Distribution and percentages of non-protein bound contraceptive steroids in human serum. J Steroid Biochem 1982;17:375-80.
9. Jenkins N, Limpongsanurak S, Fotherby K. Circulating levels of synthetic steroids in women using a triphasic formulation: a comparison with different ethinylestradiol doses. J Obstet Gynaecol 1981;2:37-40.
10. Back DJ, Bates M, Breckenridge AM, et al. The pharmacokinetics of levonorgestrel and ethinylestradiol in women-studies with Ovran and Ovranette. Contraception 1981;23:229-39.
11. O'Malley K, Stevenson I, Crooks J. Impairment of human drug metabolism by oral contraceptive steroids. Clin Pharmacol Ther 1972;13:552-7.
12. Herz R, Koelz HR, Haemmerli UP, Benes I, Blum AL. Inhibition of hepatic demethylation of aminopyrine by oral contraceptive steroids in humans. Eur J Clin Invest 1978;8:27-30.
13. Tornatore KM, Kanarkowski R, McCarthy TL, Gardner MJ, Yurchak AM, Jusko WJ. Effect of chronic oral contraceptive steroids on theophylline disposition. Eur J Clin Pharmacol 1982;23:129-34.

14. Larsson-Cohn U, Fahraeus L, Wallentin L, Zador G. Effects of the estrogenicity of levonorgestrel/ethinylestradiol combinations on the lipoprotein status. Acta Obstet Gynecol Scand Suppl 1982;105:37-40.
15. Gaspard UJ, Romus MA, Gillain D, Duvivier J, Demey-Ponsart E, Franchimont P. Plasma hormone levels in women receiving new oral contraceptives containing ethinyl estradiol plus levonorgestrel or desogestrel. Contraception 1983;27:577-90.
16. Lepot MR, Gaspard UJ. Metabolic effects of two low-dose triphasic oral contraceptives containing ethinylestradiol and levonorgestrel or gestodene. Int J Fertil 1987;32:15-20.
17. Limpongsanurak S, Fotherby K. Effect of contraceptive steroids on serum levels of sex hormone binding globulin and ceruloplasmin. Curr Med Res Opinion 1981;7:185-91.
18. Victor A, Weiner E, Johannson EDB. Relation between sex hormone binding globulin and *d*-norgestrel levels in plasma. Acta Endocrinol 1977;86:430-6.

Chairman: J. W. Goldzieher

Pharmacologic and pharmacokinetic characteristics of norgestimate and its metabolites

John L. McGuire, PhD, Audrey Phillips, PhD, DoWon Hahn, PhD, Edward L. Tolman, PhD, Soledad Flor, PhD, and Michael Edwin Kafrissen, MD, MSPH
Raritan, New Jersey

Biotransformation, pharmacologic, and pharmacokinetic studies of norgestimate and its metabolites indicate that 17-deacetyl norgestimate, along with the parent drug, contributes to the biologic response. The postulated metabolic pathway, which is based on the identification of urinary products had indicated that three metabolites of norgestimate, 17-deacetyl norgestimate, 3-keto norgestimate, and levonorgestrel, might participate in the response. The pharmacologic evaluation of these metabolites demonstrates that only 17-deacetyl norgestimate has a pharmacologic profile consistent with that of norgestimate, and significant concentrations of this metabolite have been measured in the serum of women after the administration of norgestimate. These studies indicate that 17-deacetyl norgestimate contributes to the pharmacologic response to norgestimate. (Am J Obstet Gynecol 1990;163:2127-31.)

Key words: Norgestimate, norgestimate metabolites, 17-deacetyl norgestimate

Norgestimate is a progestin used in combination with ethinyl estradiol as an oral contraceptive. Because some steroids exert their pharmacologic effects through active metabolites, in addition to the parent drug, we have investigated whether the pharmacologic activity of norgestimate (Fig. 1) might be mediated, at least in part, through any of its metabolites. Biotransformation studies have been conducted to examine the metabolism of norgestimate; the pharmacologic profiles of three metabolites have been compared with that of norgestimate, and the pharmacokinetics of norgestimate and 17-deacetyl norgestimate, a metabolite believed to contribute significantly to the biologic response, have been examined. In this article the results of these studies are reviewed, with emphasis placed on how they contribute to our further understanding of norgestimate's action.

From the R. W. Johnson Pharmaceutical Research Institute. Reprint requests: Audrey Phillips, PhD, R. W. Johnson Pharmaceutical Research Institute, Route 202, Box 300, Raritan, NJ 08876.
6/0/23622

Pharmacokinetics and biotransformation

The pharmacokinetics of ^{14}C-norgestimate have been studied in several animal species and appear to be consistent with those of other contraceptive progestins.[1] In rats, dogs, and monkeys, orally administered ^{14}C-norgestimate is rapidly absorbed, with peak levels of radioactivity achieved within 4 hours of administration, and it is eliminated with a half-life that ranges from 30 to 67 hours. After oral administration to women, ^{14}C-norgestimate is rapidly absorbed, with peak levels of radioactivity ranging from 30 minutes to 2 hours, and

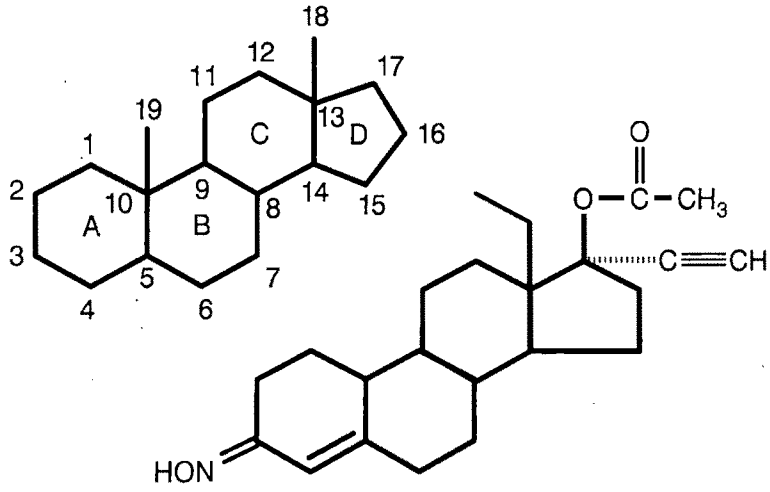


Fig. 1. Structure of norgestimate and steroid nucleus.

it is eliminated with a half-life ranging from 45 to 71 hours.[2]

The biotransformation of norgestimate in women has been investigated by identifying urinary metabolites after the oral administration of ^{14}C-norgestimate.[3] As in studies of other steroids, the identification and quantification of terminal norgestimate metabolites represent a sampling, depending on the availability of reference standards. Although these metabolites and their amounts do not necessarily reflect the total metabolite profile or their contribution to the biologic response, the chemical structures of identified urinary metabolites provide a basis to postulate the major metabolic pathways. After extraction and chromatographic separation, five 17α-ethinyl metabolites or norgestimate have been identified by gas chromatography–mass spectrometry and by comparison with several available reference standards.[3] The chemical structures of these terminal metabolites indicate that in women, norgestimate undergoes metabolism involving hydrolysis at position 17, cleavage of the oxime and subsequent reduction of the ketone at position 3, hydroxylation in the A and D rings, reduction of the double bond at C_4-C_5, and subsequent conjugation to a glucuronide or sulfate. Urinary metabolites identified in analogous studies that use ^{14}C-norgestimate in rats, dogs, and monkeys suggest that metabolic pathways in these species are similar to those in women. These studies indicate the possibility that 17-deacetyl norgestimate, 3-keto norgestimate, and 17-deacetyl–3-keto norgestimate (levonorgestrel) might contribute to the pharmacologic response observed after oral administration of norgestimate to women. To examine this possibility further, we have investigated the pharmacologic profiles of these metabolites and have compared them with that of norgestimate.

Pharmacologic evaluation of norgestimate, 17-deacetyl norgestimate, 3-keto norgestimate, and levonorgestrel

Laboratory studies. The pharmacology of norgestimate, which has been studied extensively and reported previously,[4-8] is summarized here briefly. The affinity of norgestimate for rabbit uterine cytosolic progestin receptors in vitro is similar to that of progesterone. The stability of norgestimate throughout the incubation period in the receptor-binding studies has been monitored, confirming the affinity of unchanged norgestimate for progestin receptors.[6] Additional evidence of norgestimate's inherent progestational activity is provided by experiments that show its local stimulation of the rabbit endometrium when injected directly into the uterine lumen[6] and its ability to directly inhibit luteinizing hormone–releasing hormone–stimulated luteinizing hormone release from rat pituitary cells in vitro.[8]

In conventional endocrine bioassays, norgestimate exhibits activity characteristic of a progestin. It stimulates the endometrium after oral administration to estrogen-primed immature rabbits.[5] In this test system the potency of norgestimate is maintained after subcutaneous administration (0.24 and 0.34 times that of levonorgestrel after oral and subcutaneous administration, respectively) which shows that the progestational action of norgestimate is not dependent on first-pass oral metabolism. Norgestimate, like progesterone, also maintains pregnancy in ovariectomized rats and effectively inhibits ovulation in several species.

The relative lack of androgenicity of norgestimate is shown by its very weak affinity for rat prostatic androgen receptors (relative binding affinity of 0.003 times that of dihydrotestosterone), its ability to stimulate ventral prostate growth in immature castrated rats only to the same degree as progesterone (relative potency of

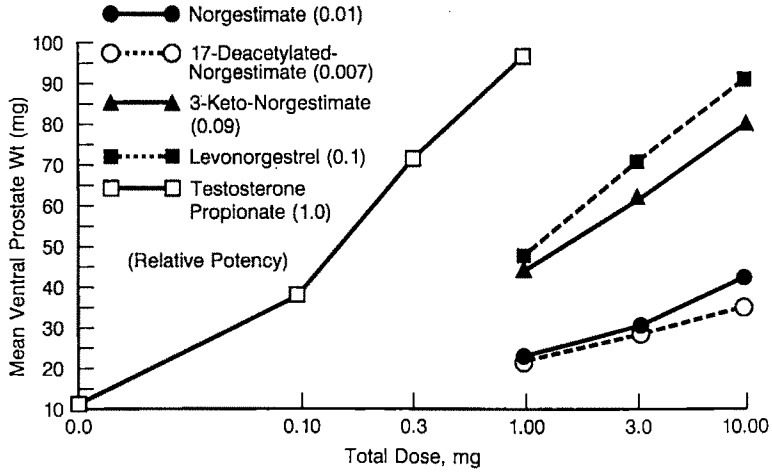


Fig. 2. Stimulation of ventral prostate growth after administration to immature castrated rats. (Relative potency estimates from Phillips et al.[6])

0.003 to 0.03 times that of testosterone),[6] and its virtual lack of affinity for human sex hormone–binding globulin (SHBG) in vitro (inhibiting concentration of 50% [IC_{50}] > 10,000 nmol/L).[7]

To determine whether 17-deacetyl norgestimate, 3-keto-norgestimate, or levonorgestrel contributes to the pharmacologic activity of the parent drug, these steroids were tested in the same assays described above. If any of these metabolites contribute significantly to the pharmacologic activity of norgestimate, their pharmacologic activity profile would be consistent with that observed after the administration of norgestimate.

17-Deacetyl norgestimate, 3-keto norgestimate, and levonorgestrel demonstrate affinity for progestin receptors by displacing ^{3}H-R5020 from rabbit uterine receptors with relative binding affinities of 0.94, 5.21, and 5.41 times that of progesterone, respectively.[6] These steroids also stimulate the endometrium of estrogen-primed immature rabbits with relative potencies of 1.4, 3.4, and 4.9 times that of norgestimate, respectively.[6] Therefore each of these three steroids show progestational activity. However, to evaluate whether they might act as biologically active metabolites of norgestimate, it was necessary to also assess their androgenicity, because a lack of androgen-related pharmacology is a characteristic or norgestimate.

Comparative laboratory assays show that levonorgestrel and 3-keto norgestimate have a pharmacologic profile different from that of norgestimate. Therefore it appears unlikely that either of these steroids contributes significantly to the pharmacologic response of norgestimate. In contrast to norgestimate, levonorgestrel has significant affinity for androgen receptors in vitro (relative binding affinity of 0.22 times that of dihydrotestosterone),[6] significant stimulation of prostate growth in rats (relative potency of about 0.1 times that of testosterone; Fig. 2),[6] and significant affinity for human SHBG in vitro (IC_{50} = 53.4 nmol/L).[7] 3-Keto norgestimate also appears to be significantly androgenic, though less so than levonorgestrel. The relative binding affinity of 3-keto norgestimate for rat androgenic receptors is 0.025 times that of dihydrotestosterone; its potency in stimulating prostate growth in rats is equal to that of levonorgestrel (Fig. 2), but this progestin exhibits no affinity for human SHBG in vitro (IC_{50} > 10,000 nmol/L).

In contrast to levonorgestrel and 3-keto norgestimate, 17-deacetyl norgestimate, like norgestimate, has little or no activity in each of the three androgenic test models. Its relative binding affinity for rat androgen receptors is only 0.013 times that of dihydrotestosterone; whereas its ability to stimulate prostate growth in rats is similar to that of norgestimate (Fig. 2). Finally, it shows a lack of affinity for human SHBG in vitro (IC_{50} > 10,000 nmol/L).

These data suggest that it is unlikely that either levonorgestrel or 3-keto norgestimate contributes significantly to the pharmacologic activity of norgestimate, since the androgenicity of these compounds is inconsistent with the pharmacologic profile of norgestimate. However, the pharmacologic profile of 17-deacetyl norgestimate is similar to that of norgestimate, which suggests that it may be an important contributing metabolite.

Clinical studies. Based on the results of preclinical studies, norgestimate and 17-deacetyl norgestimate should have minimal effect on ethinyl estradiol–induced elevations in circulating levels of SHBG and high-density lipoprotein (HDL), whereas levonorgestrel and 3-keto norgestimate should significantly reduce these levels. This prediction has been based on the sensitivity of these levels to androgenic stimulation. SHBG levels in women during combined oral contraceptive use are considered a measurement of the relative es-

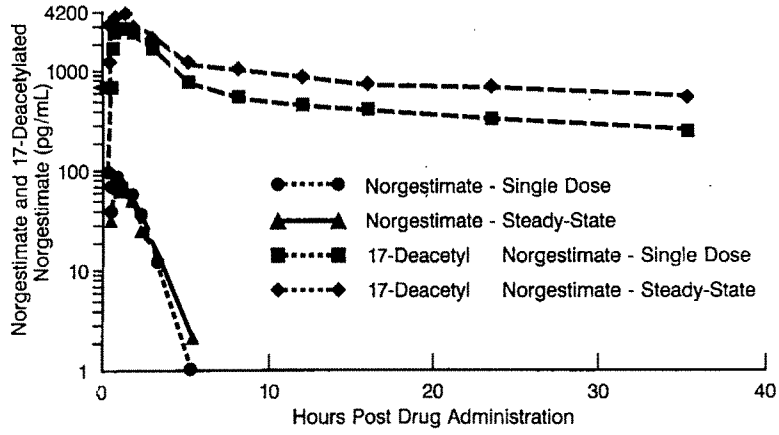


Fig. 3. Mean serum levels of norgestimate and 17-deacetyl norgestimate after single- and multiple-dose administration of 360 μg of gestimate to 10 human female subjects.

trogen and androgen activities of the combined product, since estrogens elevate and androgens lower SHBG levels.[9,10] Another androgen-sensitive parameter is lipid levels. It has been shown that estrogens elevate HDL levels whereas androgens have the opposite effect.[11-13]

Two of the progestins, norgestimate and levonorgestrel, are used in combination with ethinyl estradiol as oral contraceptives and have been compared for their effects of SHBG and HDL levels. In a study of 40 women after four treatment cycles, norgestimate does not appreciably inhibit estrogen-induced elevations in SHBG levels (161.2% change from pretreatment levels after norgestimate : ethinyl estradiol, 250 : 35 μg).[14] However, the relatively lower SHBG levels in women treated with the levonorgestrel product (26.2% change from pretreatment after levonorgestrel : ethinyl estradiol, 150 : 30 μg) indicate that levonorgestrel has markedly reduced the elevation in SHBG levels resulting from ethinyl estradiol. In a large multicenter study of these same two oral contraceptives in more than 1000 women, significant elevations in HDL levels were observed after 3, 6, 12, and 24 cycles of treatment with the norgestimate–ethinyl estradiol product (8.8% maximum increase), which is in contrast to the significant decrease in HDL levels observed during these same cycles of treatment with the levonorgestrel product (7.6% maximum decrease).[14]

These clinical data and the laboratory data demonstrate very different profiles for norgestimate and levonorgestrel, suggesting that levonorgestrel does not appear to make an important contribution to the pharmacologic activity of norgestimate.

Pharmacokinetics of norgestimate and 17-deacetyl norgestimate

The pharmacologic evaluation of norgestimate's metabolites has suggested that 17-deacetyl norgestimate might contribute to the response. Studies by Madden and Back (see this Supplement) demonstrate that 17-deacetyl norgestimate is the major metabolic product of norgestimate in human intestinal and hepatic tissues in vitro.

A recent study in our laboratory that characterized the pharmacokinetics of norgestimate and 17-deacetyl norgestimate in the serum of women after norgestimate administration is consistent with the significant contribution of this metabolite to the response to norgestimate. In this study, 10 healthy women have received single (day 1) and multiple doses (days 4 to 10) of two identical tablets, each containing 0.180 mg of norgestimate and 0.035 mg of ethinyl estradiol (the norgestimate clinical dose is 0.250 mg daily). Intensive blood sampling has been performed after the first and last doses. Serum concentrations of norgestimate and 17-deacetyl norgestimate have been measured by high-pressure liquid chromatography–radioimmunoassay procedures (unpublished data). Norgestimate is rapidly absorbed from the tablets. Mean peak norgestimate concentrations of 100 pg/ml are observed within 1 hour of administration, both after single dose and at steady state (Fig. 3), followed by a rapid decline caused by distribution and elimination. Norgestimate concentrations after single- or multiple-dose administration are generally below assay detection within 5 hours after dosing, which precludes an accurate determination of its elimination half-life.

Consistent with the in vitro studies in human intestinal and hepatic tissues, the metabolite 17-deacetyl norgestimate appears rapidly in serum after single and multiple norgestimate doses, with concentrations greatly exceeding those of norgestimate (Fig. 3). Mean peak concentrations of 3597 and 4436 pg/ml are observed at 1.5 and 1.4 hours after single dose and at steady state, respectively, which indicates that there is a rapid and substantial conversion of norgestimate to 17-deacetyl norgestimate after oral administration of

norgestimate. A certain degree of accumulation of the 17-deacetyl norgestimate is observed after multiple dosing, as would be anticipated from its estimated half-life of 16 to 17 hours.

Comment

Significant progress has been made in understanding the contribution of metabolites to the response to norgestimate. However, additional studies are needed. Other metabolites need to be identified, but it is important to point out that relatively few metabolites have been identified for any steroid to date and that such work is painstakingly difficult and time consuming. Blood levels of other metabolites also need to be determined. However, because some of these metabolites bind strongly to SHBG in serum and SHBG is significantly elevated in women using norgestimate–ethinyl estradiol, the measurements of these metabolites and the relationship of such measurements to their contribution to the response are complicated.

The studies reported here indicate that 17-deacetyl norgestimate, together with the parent drug, contributes significantly to the response to norgestimate. Significant blood levels of this metabolite have been demonstrated in women after norgestimate administration, and its pharmacology is consistent with that of norgestimate.

REFERENCES

1. Orme MLE, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokin 1983;8:95-136.
2. Weintraub HS, Abrams LS, Patrick JE, McGuire JL. Disposition of norgestimate in the presence and absence of ethinyl estradiol after oral administration to humans. J Pharm Sci 1978;67:1406-8.
3. Alton KB, Hetyei NS, Shaw C, Patrick JE. Biotransformation of norgestimate in women. Contraception 1984; 29:19-29.
4. Killinger J, Hahn DW, Phillips A, Hetyei NS, McGuire JL. The affinity of norgestimate for uterine progestogen receptors and its direct action on the uterus. Contraception 1985;32:325-32.
5. Phillips A, Hahn DW, Klimek S, McGuire JL. A comparison of the potencies and activities of progestins used in oral contraceptives. Contraception 1987;36:181-92.
6. Phillips A, Demarest K, Hahn DW, Wong F, McGuire JL. Progestational and androgenic receptor binding affinities and in vivo activity of norgestimate and other progestins. Contraception 1990;41:399-410.
7. Phillips A, Hahn DW, McGuire JL. Relative binding affinity of norgestimate and other progestins for sex hormone binding globulin. Steriods 1990;55:373-5.
8. Phillips A. The selectivity of a new progestin. Acta Obstet Gynaecol Scand 1990;152:21-4.
9. El Makhzangy MN, Wynn V, Lawrence DM. Sex hormone binding globulin capacity as an index of estrogenicity or androgenicity in women on oral contraceptive steroids. Clin Endocrinol 1979;10:39-45.
10. Victor A, Weiner E, Johansson EDB. Relationship between sex hormone binding globulin and *d*-norgestrel levels in plasma. Acta Endocrinol 1977;86:430-6.
11. Alen M, Rahkila P. Reduced high density lipoprotein cholesterol in power athletes. Use of male sex hormone derivatives, an atherogenic factor. Int J Sports Med 1984; 5:341-2.
12. Bradley DD, Wingerd J, Pettiti DB, Serum high density lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. N Engl J Med 1978; 299:17-20.
13. Fotherby K. Oral contraceptives, lipids, and cardiovascular disease. Contraception 1985;31:367-94.
14. Chapdelaine A, Desmarais JL, Derman RJ. Clinical evidence of the minimal androgenicity of norgestimate. Int J Fertil 1989;34:347-52.

Serum pharmacokinetics of orally administered desogestrel and binding of contraceptive progestogens to sex hormone–binding globulin

Willem Bergink, PhD,[a] Roel Assendorp, MSc,[a] Lenus Kloosterboer, PhD,[a] Wim van Lier, MSc,[a] Gerrit Voortman, MSc,[a] and Ingelise Qvist, MD[b]

Oss, The Netherlands, and Horsens, Denmark

Serum levels of 3-ketodesogestrel and ethinyl estradiol were analyzed by radioimmunoassay in a balanced crossover study with two tablet formulations containing desogestrel (0.150 mg) and ethinyl estradiol (0.030 mg) in 25 women under steady-state conditions after 21 days of treatment. The pharmacokinetic properties of desogestrel were characterized by the following parameters: (1) maximum serum concentration, (2) time to maximum serum concentration, (3) total area under the serum concentration versus time curve, and (4) serum half-life of elimination. The interindividual variation in these parameters was comparable with that observed with other contraceptive combinations containing ethinyl estradiol and norethisterone, levonorgestrel, or gestodene. The serum distribution of contraceptive progestogens is known to be determined by their affinity to sex hormone–binding globulin and the concentration of sex hormone–binding globulin. We analyzed the structural features that determine binding to sex hormone–binding globulin. The 13-methyl group increased and the 11-methylene group weakened the binding to sex hormone–binding globulin. The double bond at C-15 reinforced the binding only when combined with an 18-methyl group. Therefore, the binding of levonorgestrel (the 18-methyl derivative of norethisterone) and gestodene (the Δ-15,18 methyl derivative of norethisterone) to sex hormone–binding globulin was much stronger than that of 3-keto-desogestrel and norethisterone. (AM J OBSTET GYNECOL 1990;163:2132-7.)

Key words: Desogestrel, oral contraceptive, pharmacokinetics, sex hormone–binding globulin

The pharmacokinetics and metabolism of contraceptive progestogens and ethinyl estradiol (EE) have been analyzed in several studies,[1-7] and considerable variation was observed in the pharmacokinetic parameters. Only a few studies are adequate for a meaningful comparison of the rate and the extent of absorption and the rate of elimination, because in many studies data have been obtained with only a limited number of volunteers.

Parameters that are characteristic for the pharmacokinetic properties of the hormonal components in an oral contraceptive tablet, namely, maximal serum concentration (C_{max}), time to maximum serum concentration (T_{max}), area under the serum concentration versus time curve (AUC), and serum elimination half life ($T_{1/2}el$), are subject to several factors, which influence the variability. These factors result not only in considerable interindividual variation,[2,7] but also in intercenter variation in the pharmacokinetic parameters.

From the Scientific Development Group, Organon International B.V.,[a] and the Department of Obstetrics and Gynaecology, Horsens Hospital.[b]
Reprint requests: Willem Bergink, PhD, Scientific Development Group, Organon International B.V., P. O. Box 20, 5340 BH Oss, The Netherlands.
6/0/24389

Relevant to this aspect may be variations in environmental factors, patient characteristics, assay methods, and the first-pass effect. The latter was reported as specifically relevant to plasma level variability.[8]

It has also been concluded that levonorgestrel and gestodene are 80% to 100% bioavailable and therefore have no or a limited first-pass effect.[9,10] The bioavailability of other compounds (e.g., norethisterone, EE, and desogestrel) has been reported to be less. Therefore in the present study we analyzed the interindividual variability of the mean values of the pharmacokinetic parameters (C_{max}, t_{max}, AUC, and $t_{1/2}el$) for orally administered desogestrel in comparison with the values found in other studies with oral contraceptives containing EE and desogestrel, EE and levonorgestrel, EE and norethisterone, and EE and gestodene.

In addition to presystemic metabolism (i.e., absorption by the gut wall and first-pass metabolism in the liver), the plasma levels of contraceptive steroids strongly depend on binding to plasma proteins. A close correlation between plasma concentrations of levonorgestrel or norethisterone and sex hormone–binding globulin (SHBG) binding capacity as found by Victor and Johansson[11] and Back et al.[12] indicates the importance of SHBG binding for the pharmacokinetics of

contraceptive progestogens. Therefore we have also analzyed the binding affinity of contraceptive progestogens to SHBG.

Material and methods

Estimation of SHBG binding capacity. The SHBG binding capacity has been estimated with the dextran-coated charcoal method for the separation of bound and free steroid as described by Hammond and Lähteenmäki.[13] The binding was determined in the presence of cortisol (200 nmol/L) to prevent binding to transcortin-binding sites. Human serum was diluted (dilution factor 26) with a 0.25% dextran-coated charcoal, 0.025% dextran T70 suspension in buffer (10 mmol/L TRIS, 5 mmol/L $CaCl_2$, glycerol 0.3 mol/L, pH 7.4) and incubated for 30 minutes at room temperature to remove endogenous steroids. The mixture was centrifuged (15,000 N/kg for 10 minutes), and the stripped serum was used for the determination of the relative binding affinities of progestogens to SHBG.

The relative binding affinities were estimated with a 3-point parallel line assay.[14] To determine relative affinity, 0.100 ml of stripped serum and 0.100 ml of a saturating concentration of labeled 5α-dihydrotestosterone and increasing concentrations of unlabeled progestogens were incubated for 30 minutes at 37° C. Nonspecific binding was measured in the presence of 100 nmol/L of unlabeled 5α-dihydrotestosterone. The results of several tests were combined according to the procedure described in the *United States Pharmacopeia.*[15]

Estimation of pharmacokinetic parameters for EE and desogestrel. The preparations were as follows: (1) a tablet with a diameter of 6 mm, containing 0.150 mg of desogestrel + 0.030 mg of EE and (2) a smaller tablet with a diameter of 5 mm, which also contained 0.150 mg of desogestrel + 0.030 mg of EE.

Volunteers. Twenty-five healthy female volunteers, ages 20 to 29 years, who voluntarily gave written informed consent, participated in this study. The subjects were accurate with respect to tablet intake, had a cycle length around 28 days (28 ± 2 days), and had less than three abnormal-linked laboratory values during biochemical and hematologic screening before treatment.

The subjects also fulfilled the following criteria: (1) women were excluded during 3 months after a partus, an abortion, or stopping breast-feeding; (2) women should not be heavy smokers (> four cigarettes daily), heavy drinkers (not more than five glasses per week and no alcohol was taken within 3 days before an assessment day), or overweight (< ideal weight + 20%); (3) women should not have been treated with oral contraceptives or other hormonal preparations within a 2-month period before the start of the study or treated with an implant or injectable contraceptive within the previous 6 months were excluded. Moreover, women with a poor physical condition, abnormal fasting glucose levels, a history of gestational diabetes, a history or a family history of diabetes mellitus, and diseases known to be associated with lipid disorders were also excluded. Special attention was also given to the general exclusion criteria (thromboembolic disease, known or suspected estrogen-dependent tumors, etc.). Finally, subjects did not take rifampicin, tetracycline, phenylhydantoin, and phenobarbital during the 2 months before the start of treatment and during treatment.

Table I. Relative affinities of norethisterone derivatives of norethisterone for SHBG

Compound	*Relative affinity to 5α-DHT* (with 95% confidence limits)*	
Testosterone (reference)	22.0	(20.0-24.2)
NET	2.3	(2.1-2.6)
Δ-15-NET	1.3	(1.2-1.4)
11-methylene-NET	2.6	(2.4-2.9)
18-methyl-NET (levonorgestrel)	11.5	(10.2-13.0)
11-methylene,18-methyl-NET (3-ketodesogestrel)	3.9	(3.5-4.2)
11-methylene,Δ-15-NET	1.5	(1.3-1.6)
Δ-15,18-methyl-NET (gestodene)	17.3	(15.8-18.9)
11-methylene,Δ-15,18-methyl-NET	4.7	(4.3-5.1)

5α-DHT, 5α-Dihydrotestosterone; *NET,* norethisterone.
*Results of three tests were combined.

Design. The volunteers were randomly divided over two groups. Group I consisted of 11 volunteers and group II consisted of 14 volunteers.

Group I started with the 6 mm tablet and group II started with the 5 mm tablet in the first treatment cycle.

After 21 days of treatment a tablet-free period of 7 days followed, after which the treatments were reversed. Group I then received the 5 mm tablet for 21 days and group II received the 6 mm tablet for 21 days.

Blood sampling scheme. Just before and after the twenty-first dose, blood samples were taken for 3-ketodesogestrel and EE determinations. Sampling on day 21 started at 8:00 AM, just before the intake of tablet 21. The day before sampling, no food was taken after 8:00 PM. The samples were taken at the following times: 0 (just before intake), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 48 and 72 hours after intake of the tablet.

Blood was allowed to clot at room temperature for 30 minutes and then centrifuged at 2.5 × 10^4 N/kg for 10 minutes at 4° C. Serum was collected, frozen, and stored at −20° C until analysis.

EE and 3-ketodesogestrel assay. EE concentrations were estimated by a specific radioimmunoassay using 17α-[6,7-^{3}H]-EE (New England Nuclear) as tracer, specific activity 2200 gigabecquerel (GBq)/mmol, and anti-serum raised in rabbits against EE-3-succinyl–bovine serum albumin. The working dilution of the antiserum was 1/16000. The detection was accurate

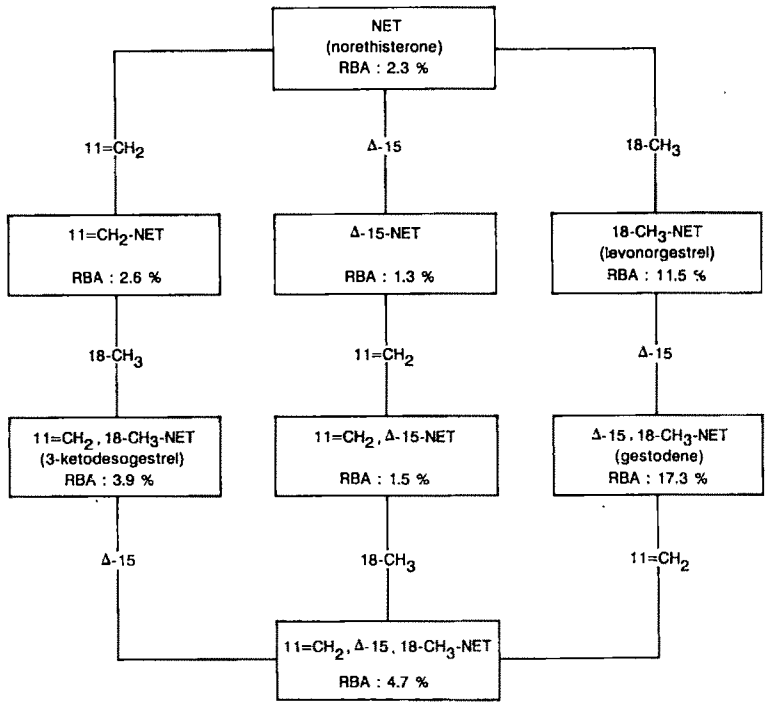


Fig. 1. Relative binding affinities *(RBAs)* of progestogens derived from norethisterone to SHBG.

with variation coefficients of <25% over the range of 20% to 80% relative binding of ligand to antibody. The sensitivity of this radioimmunoassay approximated 150 fmol/ml at 50% relative binding and 65 fmol/ml at 80% relative binding. Therefore sensitivity was sufficient for the detection of serum values of EE up to at least three elimination half-lives, namely, 180 and 100 fmol/ml were detected 48 and 72 hours, respectively, after intake of the last tablet.

3-Ketodesogestrel concentrations were estimated by a radioimmunoassay using [16,^{3}H]3-ketodesogestrel (Organon) as tracer, specific activity 1702 GBq/mmol, and antiserum raised in rabbits against 3-ketodesogestrel-3-(0-carboxymethyl)oxime–bovine serum albumin. The working dilution of the antiserum was 1/50000. Cross-reacting metabolites did not interfere, because they are not believed to be present in serum in measurable amounts after desogestrel treatment.[16] The sensitivity of this radioimmunoassay approximated 300 fmol/ml at 50% relative binding of ligand to antibody. This sensitivity was sufficient to detect the serum levels of 3-ketodesogestrel 48 and 72 hours after intake of the last tablet, namely, 1300 and 700 fmol/ml, respectively.

For both methods, before assay the samples were extracted with 3 ml "Baker SPE octadecyl (C18)" disposable columns. SAC-CEL (Wellcome Diagnostics), donkey antirabbit antiserum coupled with cellulose, was used to perform bound/free ligand separation.

Pharmacokinetic calculations. The following pharmacokinetic parameters were calculated from the 3-ketodesogestrel serum levels:

1. C_{max} and t_{max} were taken from the measured serum level data.
2. Elimination half-life ($t_{1/2}$el): by means of the individual plots of the log-concentration versus time, it was established from which time onward up to the last time the curve was approximately log-linear.
3. Using log-linear regression on these terminal data points, the elimination half-life for each individual subject was estimated.
4. AUC_0: The $AUC_{0\text{-}24}$ (= AUC of one dosing interval) was calculated by means of the linear trapezoidal rule.

Results and comment

Binding of contraceptive progestogens to SHBG. The pharmacokinetics of contraceptive progestogens are known to be influenced by the binding to SHBG. During multiple dosing of OCs containing EE and norethisterone, levonorgestrel, gestodene,[17] or desogestrel,[18] a progressive rise in the serum concentration of these progestogens or their active metabolite was found, which was correlated with the rise in SHBG.[11, 12, 17, 19] All the progestogens are bound to a variable degree to SHBG[1] as a result of a variation in their affinity to SHBG. In the present study we have estimated the binding affinities of the progestogens derived from norethisterone. Table I shows the relative

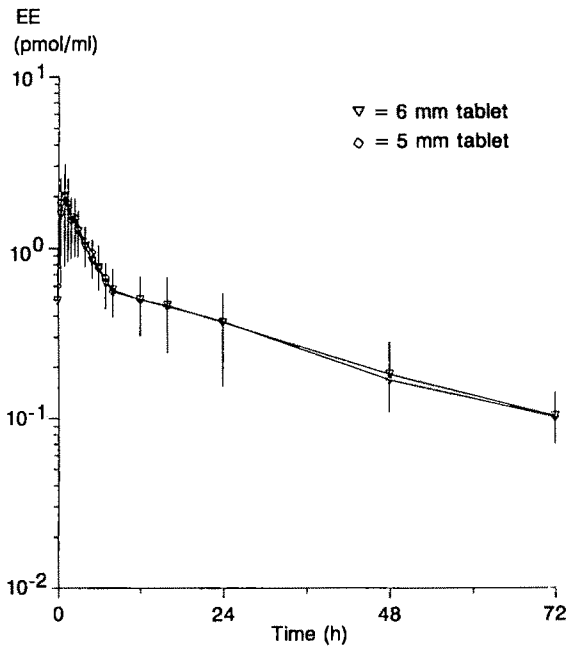


Fig. 2. Serum concentration of EE after the administration of two tablet formulations containing 0.150 mg of desogestrel and 0.030 mg of EE in 25 women on day 21 of the treatment cycle.

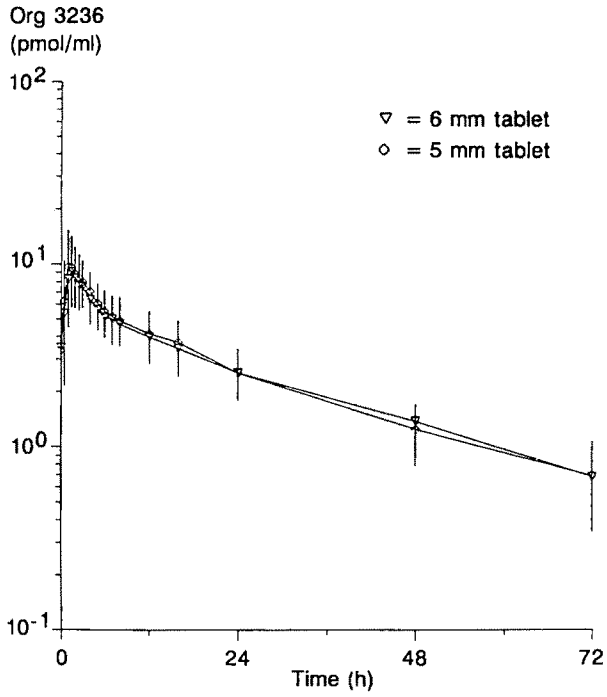


Fig. 3. Serum concentration of 3-ketodesogestrel after the administration of two tablet formulations containing 0.150 mg of desogestrel and 0.030 mg of EE in 25 women on day 21 of the treatment cycle.

binding affinities of norethisterone and the seven possible derivatives of norethisterone containing 18-methyl, 11-methylene, or Δ-15 substituents alone or combinations of these substituents. Norethisterone, levonorgestrel (18-methyl derivative), 3-ketodesogestrel (11-methylene, 18-methyl derivative), and gestodene (Δ-15, 18-methyl derivative) are presently used for contraceptive purpose. Norethisterone displays weak binding (relative binding affinity = 2.3%). The introduction of a 18-methyl group in norethisterone results in a significant increase in binding affinity (the relative binding affinity of levonorgestrel is 11.5%, as was found previously[16]), whereas the 11-methylene group and the double bond at position C-15 had no effect or resulted in a significant decrease, respectively. However, the introduction of the double bond at position C-15 (Δ-15 substitution) in levonorgestrel resulted in a further increase. The relative binding affinity of gestodene (17.3%) was only slightly less than that of testosterone (22%). The combination of the 11-methylene group and the 18-methyl group (3-ketodesogestrel) displayed a weak binding as did the combination of the double bond at position C-15 and the methylene group at C-11 (11-methylene, Δ-15-norethisterone). The compound with all three possible modifications had a weak binding to SHBG. The influence of all these modifications of the norethisterone molecule is schematically represented in Fig. 1.

Pharmacokinetics of desogestrel (0.150 mg) and EE (0.030 mg) in tablets under steady-state conditions. In the present study we have estimated serum EE and serum 3-ketodesogestrel concentrations in 25 women in a crossover bioequivalence study with two types of tablets containing 0.030 mg of EE and 0.150 mg of desogestrel, which were taken for 21 days. Volunteers were divided into two groups. One group started with the smaller tablet (5 mm), whereas the other group took the larger 6 mm tablet. Treatment was reversed after 21 days of treatment and a tablet-free period of 7 days. Blood samples were taken just before and after the last tablet of each treatment cycle (day 21) for the estimation of EE and 3-ketodesogestrel. The results demonstrated complete bioequivalence for both tablets.

Mean plasma EE and 3-ketodesogestrel concentrations after intake of both tablets at day 21 of the treatment cycles are shown in Figs. 2 and 3, respectively. The ratio of peak levels (C_{max}) and AUC in the present study are comparable with the ratios found with other combined OCs.[21, 22]

The pharmacokinetics of EE are best described by a two-compartment model as found by Hümpel et al.[23] and Back et al.[4] In the present study there was no evidence of an increase in serum EE between 10:00 AM and 2:00 PM after table intake as found by Back et al.,[24] after a single oral dose. Such an increase was considered indicative for enterohepatic circulation. Variable values

Table II. Variability in pharmacokinetic parameters of EE administered orally in combination with desogestrel (present study) or norethisterone

Author + dose	t_{max} *(hr)*	C_{max} *(pg/ml)*	$AUC_{0\text{-}24}$ *(ng · hr/ml)*	$t_{1/2}$ *(hr)*
Present study (n = 25)	1.4 ± 0.8*	739 ± 308	5.02 ± 1.57	29.1 ± 13.0
(steady state; 0.030 mg)	(cv = 57%)	(cv = 42%)	(cv = 31%)	(cv = 45%)
Stanczyk et al.[22] (n = 24)	1.5 ± 0.6	574 ± 211	4.85 ± 1.81	9.4 ± 3.18
(single dose; 0.120 mg)	(cv = 40%)	(cv = 37%)	(cv = 37%)	(cv = 33%)

From Stanczyk FZ, et al. Contraception 1983;28:241-5. Reprinted by permission of the copyright holder, Butterworth-Heinemann.

cv, Coefficient of variation.

*Values are mean ± standard deviation.

Table III. Variability in pharmacokinetic parameters of desogestrel, norethisterone, levonorgestrel, and gestodene administered orally in combination with EE

Authors + dose	t_{max} *(hr)*	C_{max} *(ng/ml)*	*AUC (ng hr/ml)*	$t_{1/2}$ *(hr)*
Present study (n = 25) (0.150 mg desogestrel	1.6 ± 1.0*	3.69 ± 0.97*	35.9 ± 7.8*	23.8 ± 5.3*
steady state with 0.030 mg EE)	(cv = 62%)	(cv = 27%)	(cv = 23%)	(cv = 22%)
Hasenack et al.[26] (n = 10) 0.150 mg desogestrel;	1.75 ± 0.75	6.36 ± 1.75	45.5 ± 24.4	ND
steady state with 0.030 mg EE)	(cv = 42%)	(cv = 28%)	(cv = 54%)	
Kuhl et al.[19] (n = 11) (0.150 mg desogestrel;	ND	ND	32.0 ± 14.3	ND
steady state with 0.030 mg EE)			(cv = 45%)	
Back et al.[27] (n = 9) (0.150 mg desogestrel; sin-	ND	ND	19.1 ± 6.5	12.4 ± 4.1
gle dose with 0.030 mg EE)			(cv = 34%)	
Stanczyk et al.[22] (n = 24) (1.0 mg norethisterone;	1.17 ± 0.65	15.7 ± 6.17	84 ± 29.4	8.1 ± 1.9
single dose with 0.120 mg EE)	(cv = 59%)	(cv = 39%)	(cv = 35%)	(cv = 24%)
Hümpel et al.[25] (n = 6) (0.150 mg levonorgestrel	≈2	3.2 ± 1.0	32 ± 10	27 ± 4.6
cv = 17%; single dose with 0.030 mg EE)		(cv = 31%)	(cv = 31%)	
Back et al.[28] (n = 9) (0.150 mg levonorgestrel;	2.4	3.8 ± 1.2	20 ± 7.8	8.0 ± 3.2
single dose with 0.030 mg EE)		(cv = 31%)	(cv = 39%)	(cv = 40%)
Täuber et al.[10] (n = 6) (0.075 gestodene; single	1.7	3.6 ± 1.4	35.0 ± 16.6	11.8 ± 2.3
dose with 0.030 mg EE)		(cv = 39%)	(cv = 47%)	(cv = 20%)

ND, Not determined; *cv,* coefficient of variation.

*Values are mean ± standard deviation.

of the $t_{1/2}$el of EE have been found in various studies.[1] For a proper estimation of the $t_{1/2}$el, serum concentrations should be followed for at least three half-lives, because a shorter observation period will result in an underestimation of the value for $t_{1/2}$el. In the present study the last point was estimated 72 hours after dosing and the corresponding $t_{1/2}$el for EE was 29.1 hours (Table II). This value was longer than the values found in most studies but was comparable with the value estimated by Hümpel et al.[25] (26 hours). The interindividual variation in the pharmacokinetic parameters characteristic for rate (t_{max}, C_{max}) and the extent of absorbtion (AUC) is considerable (coefficient variance about 30% to 40%; Table II). This value is comparable with the variability in the parameters for EE as found in the study performed by Stanczyk et al.,[22] who used a similarly large number of volunteers (Table II). The mean serum level versus time profile for 3-ketodesogestrel after the administration of 0.150 mg of desogestrel in combination with 0.030 mg of EE is shown in Fig. 3. Mean peak levels of 3.69 ng/ml were attained within 1 to 2 hours (1.6 hours) after dosing. The calculated AUC was 35.9 ng/ml, which closely resembles the AUC found by Kuhl et al.[19] (32.0 ± 14.3). The serum level variability of 3-ketodesogestrel in the present study is of the same order of magnitude as the variability in serum levels of norethisterone,[22] levonorgestrel,[24, 25] and gestodene[10] (Table III).

It has been suggested by Goldzieher[8] that variability in plasma levels should be attributed to differences in first-pass effect. The present results indicate that the intersubject variability in the pharmacokinetics of desogestrel was comparable with that of levonorgestrel, norethisterone, and gestodene. The bioavailability of levonorgestrel[9] and gestodene[10] has been reported to be nearly 100%, whereas the bioavailability of norethisterone,[29] EE,[5] and desogestrel[27] was less. The variability in serum levels for all these compounds was, however, comparable. Therefore we conclude that the comparable variability cannot be related to possible differences in first-pass effects.

REFERENCES

1. Orme MLE, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1983;8:95-136.
2. Fotherby K. Variability of pharmacokinetic parameters

for contraceptive steroids. J Steroid Biochem 1983;19: 817-20.
3. Goebelsmann U. Pharmacokinetics of contraceptive steroid in humans. In: Gregoire AI, Blye RP, eds. Contraceptive steroids: pharmacology and safety. New York: Plenum Press, 1986:67-111.
4. Back DJ, Breckenridge AM, Crawford FE, et al. An investigation of the pharmacokinetics of ethinyloestradiol in women using radioimmunoassay. Contraception 1979; 20:263-73.
5. Newburger J, Goldzieher J. Pharmacokinetics of ethinyloestradiol: a current view. Contraception 1985;32:33-44.
6. Brenner PF, Goebelsmann U, Stanczyk FZ, Mishell DR. Serum levels of ethinylestradiol following its ingestion alone or in oral contraceptive formulations. Contraception 1980;22:85-95.
7. Stadel BC, Sternthal PM, Schlesselman JJ, et al. Variation of ethinylestradiol blood levels among healthy women using oral contraceptives. Fertil Steril 1980;33:257-60.
8. Goldzieher JW. Progestogen pharmacokinetics and the unknown. Int Proc J 1989;1:21-5.
9. Stanczyk FZ. Pharmacology, pharmacokinetics and pharmacodynamics; pharmacokinetics of progestogens. Int Proc J 1989;1:11-20.
10. Täuber U, Tack JW, Matthes H. Single dose pharmacokinetics of gestodene in women after intravenous and oral administration. Contraception 1989;40:461-79.
11. Victor A, Johansson EDB. Effects of d-norgestrel induced decreases in sex hormone binding globulin capacity on the d-norgestrel levels in plasma. Contraception 1977; 16:115-23.
12. Back DJ, Breckenridge AM, Crawford FE, et al. The pharmacokinetics of norethindrone in women (1). Radioimmunoassay and concentrations during multiple dosing. Clin Pharmacol Ther 1978;24:439-47.
13. Hammond GL, Lähteenmäki PLA. A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin capacities. Clin Chem Acta 1983;132:101-10.
14. Rodbard D, Lewald JE. Computer analysis of radio ligand assay and radio immuno assay data. Acta Endocrinol Copenh Suppl 1970;147:79-103.
15. United States Pharmacopeia XXII Revision. Rockville, Maryland: Pharmacopeial Convention Inc, 1990:1510-1.
16. Shaw MA, Back DJ, Crowie AM, Orme MLE. A nonchromatographic radioimmunoassay for 3-oxodesogestrel. J Steroid Biochem 1984;22:111-7.
17. Kuhl H, Jung-Hoffmann C, Heidt F. Alterations in the serum level of gestodene and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 75 μg gestodene. Contraception 1988;38:477-86.
18. Viinikka L, Ylikorkala O, Vikho R, Wijnand HP, Booy M, van der Veen F. Metabolism of a new synthetic progestagen, Org 2969, in female volunteers. Pharmacokinetics after an oral dose. Eur J Clin Pharmacol 15, 1979;349-55.
19. Kuhl H, Jung-Hoffman C, Heidt F. Serum levels of 3-ketodesogestrel and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 150 μg desogestrel. Contraception 1988;38:381-90.
20. Bergink EW, Hamburger AD, de Jager E, van der Vies J. Binding of a contraceptive progestogen Org 2969 and its metabolites to receptor proteins and human sex hormone binding globulin. J Steroid Biochem 1981;14:175-83.
21. Goldzieher JW, Dozier TS, de la Pena A. Plasma levels and pharmacokinetics of ethinyl estrogens in various populations I Ethynyl estradiol. Contraception 1980;21:1-15.
22. Stanczyk FZ, Mroszczak EJ, Ling T, et al. Plasma levels and pharmacokinetics of norethindrone and ethinyl estradiol administered in solution and as tablets to women. Contraception 1983;28:241-51.
23. Hümpel M, Nieuweboer B, Wendt H, Speck U. Investigations of pharmacokinetics of ethinyloestradiol to specific consideration of a possible first-pass effect in women. Contraception 1979;19:421-32.
24. Back DJ, Bates M, Breckenridge AM, et al. The pharmacokinetics of levonorgestrel and ethinyloestradiol in women—studies with Ovran and Ovranette. Contraception 1981;23:229-39.
25. Hümpel M, Wendt H, Pommerenke G, Weiss C, Speck W. Investigations of pharmacokinetics of levonorgestrel to specific considerations of a possible first-pass effect in women. Contraception 1979;17:207-20.
26. Hasenack HG, Bosch AMG, Käär K. Serum levels of 3-ketodesogestrel after oral administration of desogestrel and 3-ketodesogestrel. Contraception 1986;33:591-6.
27. Back DJ, Grimmer SFM, Shenoy N, Orme MLE. Plasma concentrations of 3-ketodesogestrel after oral administration of desogestrel and intravenous administration of 3-ketodesogestrel. Contraception 1987;35:619-26.
28. Back DJ, Killick SR, Stevenson PJ, Shenoy N, Elstein M, Cohen M. The relative bioavailability of levonorgestrel and ethinyl oestradiol when administered in tablet and capsule form. Contraception 1987;36:321-6.
29. Back DJ, Breckenridge AM, Crawford FE, MacIver M, Orme MLE, Rowe PH, Smith E. The pharmacokinetics of norethindrone in women (2). Single dose pharmacokinetics. Clin Pharmacol Ther 1978;24:448-53.

SESSION II. GASTROINTESTINAL METABOLISM OF ORAL CONTRACEPTIVE STEROIDS

Chairman: J. Hammerstein

Gastrointestinal metabolism of contraceptive steroids

David J. Back, PhD, Steven Madden, BSc, and Michael L'E. Orme, MD
Liverpool, England

A number of oral contraceptive steroids undergo first-pass metabolism in the gastrointestinal mucosa. Ethinyl estradiol (mean systemic bioavailability 40% to 50%) is extensively metabolized, principally to a sulfate conjugate. In vivo studies that use portal vein catheterization and the administration of radiolabeled ethinyl estradiol have shown that the fraction of steroid metabolized in the gut wall is 0.44. In vitro studies with jejunal biopsy samples or larger pieces of jejunum or terminal ileum mounted in Ussing chambers have indicated that more than 30% of added ethinyl estradiol is sulfated. The progestogen desogestrel is a prodrug that is converted to the active metabolite 3-ketodesogestrel. Substantial first-pass metabolism of desogestrel occurs in the gut mucosa, with evidence from Ussing chamber studies for the formation of the active metabolite. Another progestogen, norgestimate, is also metabolized by the gut wall in vitro of which the principal metabolite is the deacetylated product, norgestrel oxime. It seems very likely that this will also occur in vivo. Drug interactions occurring in the gut wall have been reported with ascorbic acid (vitamin C) and paracetamol. (Am J Obstet Gynecol 1990;163:2138-45.)

Key words: Intestinal metabolism, contraceptive steroids, ethinyl estradiol, desogestrel, norgestimate

The first suggestion that estrogens were metabolized in the gastrointestinal mucosa came from studies performed by Lehtinen et al.[1, 2]; they showed the formation of a glucuronide conjugate of 17β-estradiol in rat duodenal slices. Further studies in the rat confirmed the formation of this metabolite[3] and also demonstrated the metabolism of estriol to the 16- and 17β-monogluconides. At the same time, Diczfalusy et al.,[4, 5] demonstrated the formation of glucuronide conjugates of estriol and estrone in patients either with a biliary fistula or who required surgery on the small intestine and in whom an isolated loop of small intestine was formed. A number of other studies on the gut wall metabolism of natural estrogens were subsequently reported, and these have been reviewed by Adlercreutz et al.[6] Interest in the possible intestinal metabolism of synthetic estrogens (and progestogens) has been much more recent. The stimulus to work in this area was the finding that ethinyl estradiol (EE_2) was well absorbed in women but had only a mean systemic bioavailability of 40% to 50%.[7] The possibility of presystemic metabolism in both the gut wall and the liver had to be considered. This article summarizes studies, both in vitro and in vivo, on the gut wall metabolism of EE_2 in humans. The intestinal metabolism of the progestogens desogestrel and norgestimate is also considered.

From the Department of Pharmacology and Therapeutics, University of Liverpool.
Reprint requests: David J. Back, PhD, Department of Pharmacology and Therapeutics, University of Liverpool, P. O. Box 147, Liverpool, L69 3BX, England.
6/0/23823

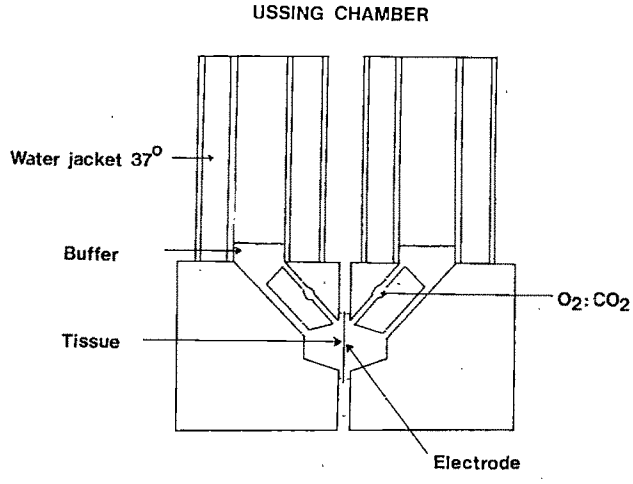


Fig. 1. Diagram of Ussing chambers.

Intestinal metabolism of EE_2

In vivo studies: Portal vein catheterization. Five patients (ages 58 to 70 years; three men and two women) who had recently undergone surgery for carcinoma of the colon and had then received 5-flurouracil administration into the portal vein by a cannula inserted through the remnant of the umbilical vein participated in the study.[8] After an overnight fast, a single tablet of

Table I. Area under plasma radioactivity versus time curve for the diffference between portal and peripheral venous samples for nonether extractable (conjugates) and total radioactivity; third column shows fraction of drug metabolized in the gut wall on first pass; subjects were given an oral dose of 50 μg (65 μCi) EE_2

Subject	$AUC_{HP.M} - AUC_{V.M}$ *(dpm · m⁻¹ × min)*	$AUC_{HP.T} - AUC_{V.T}$ *(dpm · m⁻¹ × min)*	*Fraction of drug metabolized in the gut wall (fm_G)*
1	53,040	85,125	0.62
2	14,915	52,815	0.28
3	22,055	61,775	0.40
4	106,370	225,610	0.47
5	70,985	161,745	0.44
		mean ± SE	0.44 ± 0.05

From Back DJ, Breckenridge AM, MacIver M, et al. The gut wall metabolism of ethinyloestradiol and its contribution to the pre-systemic metabolism of ethinyloestradiol in humans. Br J Clin Pharmacol 1982;13:325-30.

fm_G, First pass.

Table II. Metabolism of EE_2 by human small intestine; EE_2 and conjugates were determined in mucosal and serosal chambers 2 hours after addition of [^{3}H]-EE_2 (2 μCi; 80 ng) to the mucosal chamber

		% of added drug present as		
	% net transport	*Unconjugated steroid*	*Sulfates*	*Glucuronides*
Mucosal	—	56.6 ± 11.4	33.3 ± 12.4	2.1 ± 2.3
Serosal	2.3 ± 0.8	0.4 ± 0.3	0.7 ± 0.5	0.9 ± 0.8

Reprinted from Rogers SM, Back DJ, Orme ML'E. Intestinal metabolism of ethinyloestradiol and paracetamol in vitro: studies using Ussing chambers. Br J Clin Pharmacol 1987;23:727-34.

Results are mean ± SD (n = 10) of tissues from five patients.

Eugynon 50 (50 μg EE_2 incorporationg 65 μCi ^{3}H-EE_2; 500 μg norgestrel) was given by mouth. Before dosing and at 10, 20, 30, 40, 50, and 60 minutes after dosing, blood samples were taken simultaneously from the portal vein cannula and from a cannula inserted into a forearm vein. Total radioactivity and ether extractable radioactivity (before and after hydrolysis with β-glucuronidase/sulfatase enzyme) were determined.

The following equations for the rate of drug absorption across the gastrointestinal mucosa (dAa/dt) and the rate of metabolism of EE_2 by the mucosa (dAm/dt) were used to calculate the fraction of EE_2 metabolized.

$$\frac{dAa}{dt} = Q_{HP} \cdot C_{HP.T} - Q_{HP} \cdot C_{A.T} \qquad (1)$$

where Q_{HP} is the blood flow in the hepatic portal vein, $C_{HP.T}$ is the concentration of total radioactivity in the hepatic portal vein, and $C_{A.T}$ is the concentration of total radioactivity in the mesenteric artery.

$$\frac{dAm}{dt} = Q_{HP} \cdot C_{HP.M} - Q_{HP} \cdot C_{A.M} \qquad (2)$$

where $C_{HP.M}$ is the concentration of metabolite in the hepatic portal vein and $C_{A.M}$ is the concentration of metabolite in the mesenteric artery.

With the assumption that $C_{A.T} = C_{V.T}$ and $C_{A.M} = C_{V.M}$, where $C_{V.T}$ and $C_{V.M}$ are the concentration of total radioactivity or metabolite in the peripheral vein, integration of equations 1 and 2 yields:

Amount of total drug absorbed

$$(D) = Q_{HP}\,(AUC_{HP.T} - AUC_{V.T})$$

and

Amount of conjugate absorbed

$$(A_M) = Q_{HP}\,(AUC_{HP.M} - AUC_{V.M})$$

Therefore the fraction of EE_2 metabolized in the gut wall on the first pass (fm_G)

$$fm_G = A_{M/D}\frac{(AUC_{HP.M} - AUC_{V.M})}{AUC_{HP.T} - AUC_{V.T})}$$

The mean fraction of EE_2 metabolized in the gut wall was 0.44 (Table I). This was nearly twice as much as that in the liver (0.25).

Clearly there are certain limitations in this portal vein study, in that the patients were elderly, had first undergone major surgery, and had also received 5-fluouracil. However, these factors might be expected to reduce rather than enhance intestinal metabolism.

Further supportive evidence for the importance of intestinal sulfation has come from a study in which plasma concentrations of EE_2 and EE_2-sulfate were de-

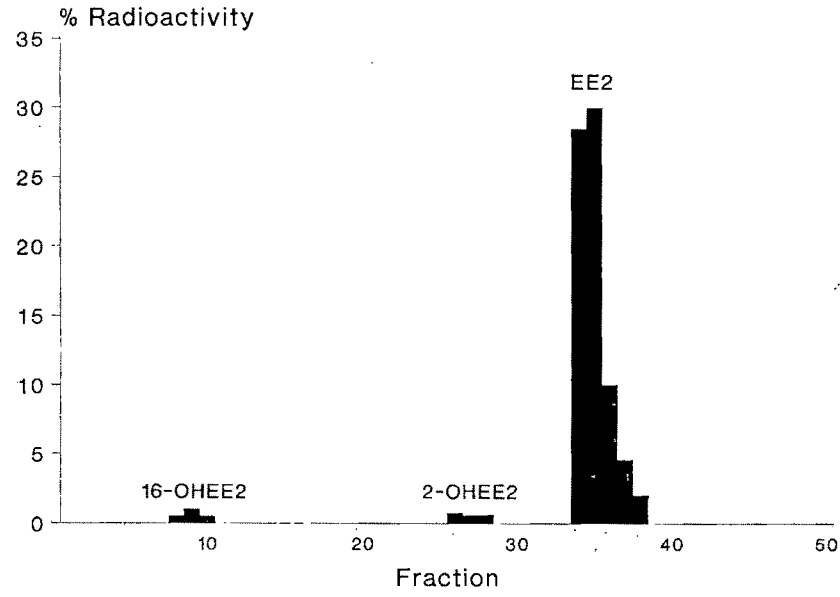


Fig. 2. A representative high-performance liquid chromatograph of an ether extract of mucosal fluid in a study with [^{3}H]-EE_2. Peak assignments are based on co-chromatography with authentic unlabeled steroids. Similar profiles were also obtained after hydrolysis with sulfatase/β-glucuronidase enzyme, indicating the presence of small amounts of oxidative metabolites in both unconjugated and conjugated fractions. (Modified from Rogers SM, Back DJ, Orme ML'E. Intestinal metabolism of ethinyloestradiol and paracetamol in vitro: studies using Ussing Chambers. Br J Clin Pharmacol 1987;23:727-34.)

termined after intravenous and oral administration of EE_2.[9] After oral dosing, the area under the curve (AUC) from 0 to 24 hours of plasma EE_2-sulfate was 18 times higher than the AUC of EE_2. After intravenous administration, the AUC of the sulfate was only six times greater than that of EE_2. These data are consistent with extensive first-pass metabolism.

In vitro studies with human jejunal biopsies. Jejunal biopsy samples were obtained from 20 subjects by means of the Quinton hydraulic biopsy sampler.[10] Samples were incubated with radiolabeled EE_2 in Krebs-Henseleit buffer for 1½ hours at 37° C. After ether extraction, the aqueous phases were subjected to hydrolysis by sulfatase in the absence and presence of glucaro-1,4-lactone. Conjugation was extensive (38% of added EE_2 was nonether extractable after incubation), and sulfation appeared to be more important than glucuronidation. Conjugates were of parent EE_2, with the extent of conjugation directly related to the weight of biopsy tissue.

It was of interest that in a parallel study with mestranol (the 3-methyl prodrug of EE_2) and levonorgestrel, only approximately 5% of both drugs was conjugated.

In vitro studies with Ussing chambers. Histologically normal jejunum or terminal ileum was obtained from five patients undergoing resections for localized tumors, diverticulosis, and Crohn disease.[11] While mounted on a glass tube, the muscularis externa was stripped off the mucosa, and then after a longitudinal incision, the mucosal sheets were mounted between two perspex chambers (Ussing chambers; Fig. 1). After the addition of radiolabeled EE_2 (2 μCi; 80 ng) to the mucosal chamber (total volume 2.5 ml of Krebs-Henseleit buffer at 37° C), sampling was undertaken from both the mucosal and serosal chambers at intervals to 2 hours. Aliquots of mucosal and serosal fluid were extracted with ether for the determination of unconjugated steroid. Aliquots of the remaining aqueous phase were incubated for 2 hours at 37° C with β-glucuronidase/sulfatase enzyme either in the presence or absence of glucaro-1,4-lactone. After extraction of liberated steroid, extracts were evaporated to dryness, and the residue was dissolved in a small volume of methanol for high-performance liquid chromatographic analysis.[12]

The overall net transport of steroid from the mucosal chamber to the serosal chamber in 2 hours was low (2.3% ± 0.8%), with the presence in the serosal chamber at that time of unconjugated (free) steroid (0.4% ± 0.3%), sulfate conjugates (0.7% ± 0.5%), and glucuronide conjugates (0.9 ± 0.8%) (Table II). In the mucosal chamber 56.6% ± 11.4% of the added drug was present as unconjugated steroid, 33.3% ± 12.4% as sulfate conjugates, and 2.1% ± 2.3% as glucuronides.

High-performance liquid chromatographic analysis of the mucosal fluid revealed some evidence of trace amounts of phase I hydroxylated metabolites (Fig. 2).

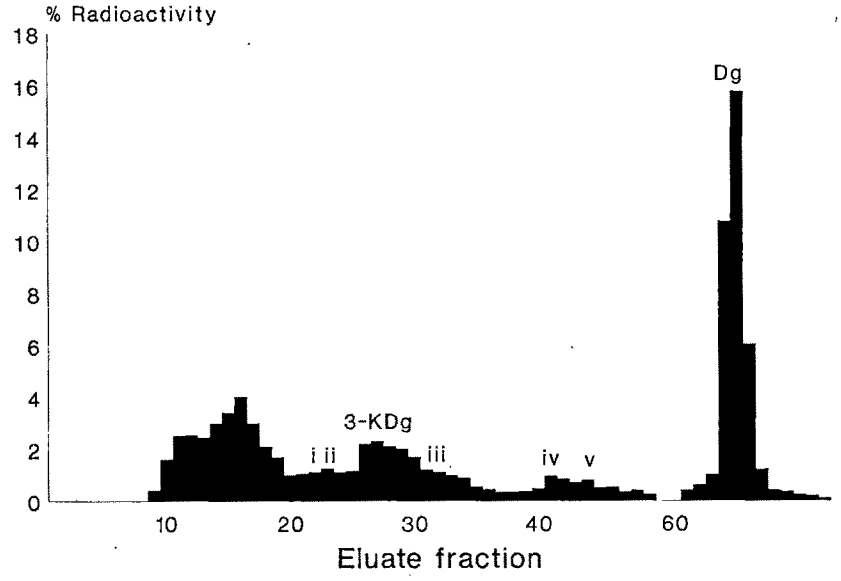


Fig. 3. Representative high-performance liquid chromatograph of an ether extract of mucosal fluid in a study with [^{3}H]-desogestrel. Solvent system was acetonitrile/water (55/45), which increased after 15 minutes to 100% acetonitrile over a 5-minute linear gradient, followed by a 30-minute isocratic elution. Peak assignment is based on co-chromatography with authentic unlabeled standards. *i*, 3β-Hydroxy desogestrel; *ii*, 3β-hydroxy, 5α-dihydro desogestrel; *iii*, 3α-hydroxy desogestrel; *iv*, 3α-hydroxy, 5α-dihydro desogestrel; *v*, 3-keto, 5α-dihydro desogestrel. (Modified from Madden S, Back DJ, Martin CA, Orme ML'E. Metabolism of the contraceptive steroid desogestrel by the intestinal mucosa. Br J Clin Pharmacol 1989;27:295-9.)

Table III. Metabolism of desogestrel by human colon (patients 1 to 6) or ileum (patients 7 and 8); desogestrel and metabolites were determined in the mucosal chamber 2 hours after addition of [^{3}H]-desogestrel (0.2 μCi; 100 ng) to the mucosal chamber

		% of drug present in mucosal chamber as		
Patient	*Desogestrel*	*Phase I metabolites*	*Sulfates*	*Glucuronides*
1 (F; 81 yr)	61.4	20.2	17.0	1.4
2 (M; 67 yr)	51.8	36.2	10.7	1.3
3 (M; 57 yr)	64.6	23.5	10.6	1.3
4 (M; 57 yr)	59.2	26.3	12.5	3.4
5 (M; 65 yr)	33.3	48.6	15.3	2.9
6 (M: 57 yr)	62.3	18.8	14.0	4.9
Mean ± SD	55.4 ± 11.7	28.9 ± 11.4	13.3 ± 2.6	2.5 ± 1.5
7 (F; 82 yr)	65.8	8.2	24.2	1.8
8 (F; 33 yr)	53.4	36.9	5.6	4.3

From Madden S, Back DJ, Martin CA, Orme ML'E. Metabolism of the contraceptive steroid desogestrel by the intestinal mucosa. Br J Clin Pharmacol 1989;27:295-9.

These observations, which indicated in excess of 30% of EE_2 was sulfated, are in agreement with the study[10] that used small pieces of mucosa obtained at biopsy and with the findings of in vivo studies.

Thus it appears that there is a difference between the intestinal metabolism of endogenous estrogens, which predominantly form glucuronide conjugates,[4, 5] and synthetic EE_2, which is mainly sulfated.

Intestinal metabolism of desogestrel

Desogestrel (13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20yn-17ol) is effectively a prodrug and is metabolized to the active metabolite 3-ketodesogestrel.[13] In a pharmacokinetic study of nine subjects, Back et al.[14] demonstrated that the mean (±SD) bioavailability of 3-ketodesogestrel was 76% ± 22% (range 40% to 113%) after the oral administration of desogestrel to volunteers who also received intravenous 3-keto desogestrel. The possibility that intestinal metabolism was involved in the first-pass metabolism of desogestrel has been examined in studies with Ussing chambers.[15]

Histologically normal ileum or colon was obtained from eight patients undergoing resections for localized

Table IV. Effect of EE_2 (0.1, 1.0, and 10 μg) present in the mucosal chamber on the metabolism of desogestrel by human colon; desogestrel and metabolites were determined 2 hours after addition of [^{3}H]desogestrel (0.2 μCi; 100 ng) to the mucosal chamber

	% of drug present in the mucosal chamber			
	No EE_2	*0.1 μg*	*1.0 μg*	*10 μg*
Parent	55.4 ± 11.7	58.7 ± 13.5	52.3 ± 20.3	53.4 ± 22.1
Phase I	28.9 ± 11.4	29.5 ± 12.5	38.5 ± 19.4	35.3 ± 20.9
Sulfates	13.3 ± 2.6	9.1 ± 2.6*	6.8 ± 2.1†	6.9 ± 2.6†
Glucuronides	2.5 ± 1.5	2.4 ± 1.2	1.7 ± 0.5	1.7 ± 1.5

From Madden S, Back DJ, Martin CA, Orme ML'E. Metabolism of the contraceptive steroid desogestrel by the intestinal mucosa. Br J Clin Pharmacol 1989;27:295-9.

Mean ± SD (n = 6).

Significantly different from control (no EE_2).

*$p < 0.05$.

†$p < 0.01$.

Table V. Effect of paracetamol (either 50 μg or 1 mg present in the mucosal chamber) on the metabolism of EE_2 by human small intestine; EE_2 and conjugates were determined 2 hours after addition of [^{3}H]-EE_2 (2 μCi; 80 ng) to the mucosal chamber

	Added drug (%) present in mucosal chamber		
		Paracetamol	
	No paracetamol	*50 μg*	*1 mg*
Unconjugated EE_2	56.6 ± 11.4	67.0 ± 9.6	88.1 ± 10.1†
Conjugates (total)	35.3 ± 11.4	24.4 ± 6.9*	10.0 ± 9.0†
Sulfates	33.3 ± 12.4	22.4 ± 6.3*	6.8 ± 4.6†
Glucuronides	2.1 ± 2.3	2.0 ± 2.7	3.1 ± 5.0

From Rogers SM, Back DJ, Orme ML'E. Intestinal metabolism of ethinyloestradiol and paracetamol in vitro: studies using Ussing chambers. Br J Clin Pharmacol 1987;23:727-34.

Results are mean ± SD of tissues (n = 10) from five patients for the controls, of tissues (n = 7) from three patients for paracetamol (50 μg), and of tissues (n = 10) from three patients for paracetamol (1 mg).

Since in the presence of paracetamol <1% of steroid was transported to the serosal side, data are presented for drug present in mucosal samples.

Significantly different from control.

*$p < 0.05$.

†$p < 0.001$.

tumors. Mucosal sheets were prepared and mounted as previously described. Radiolabeled desogestrel ([^{3}H]-desogestrel; 0.2 μCi; 100 ng) was added to the mucosal chamber. Two hours after the addition of [^{3}H]-desogestrel more than 90% of the steroid was present in the mucosal chamber. In studies with the colon, metabolite analysis (by differential enzyme hydrolysis followed by high-performance liquid chromatography) showed that 55.4% ± 11.7% (mean ± SD; N = 6) of the drug present was desogestrel, 28.9% ± 11.4% as unconjugated phase I metabolites, 13.3% ± 2.6% as sulfate conjugates, and 2.5% ± 1.5% as glucuronide conjugates (Table III).

By co-chromatography with authentic metabolites and mass spectrometry, it was shown that the active metabolite (3-ketodesogestrel) was formed in the mucosa (Fig. 3). A large peak of the radioactivity did not co-chromatograph with any known metabolites and was tentatively identified as ring hydroxylated products of 3-ketodesogestrel.

In comparison with the previously described studies with EE_2 several interesting points emerge. First, unlike EE_2, which formed only trace amounts of hydroxylated metabolites, desogestrel apparently undergoes fairly extensive oxidative metabolism. It has been previously reported that in humans the liver:intestine ratio of cytochrome P-450 is approximately 20[16] and that cytochrome P-450–dependent phase I biotransformations in the intestine is unlikely to contribute substantially to the overall metabolism of drugs in humans. Although it is difficult to draw conclusions regarding the contribution of the gut to first-pass metabolism of desogestrel in vivo, the metabolic profiles obtained in vitro give clear evidence of the potential of the gut to metabolize the steroid. Second, the formation of sulfate conjugates in preference to glucuronide conjugates

was evident with both EE_2 and desogestrel. We followed this point up in a study in which we examined the effect of adding increasing concentrations of EE_2 (100 ng, 1 and 10 μg) to Ussing chambers containing radiolabeled desogestrel (i.e., a situation analogous with in vivo, in which the two steroids are present in the combined oral contraceptive (OC). Desogestrel sulfation was reduced by 32%, 49%, and 48% in the presence of 0.1, 1, and 10 μg of EE_2, respectively (Table IV). The D rings of desogestrel and EE_2 are identical, and therefore it is possible that substrate competition exists for the same sulfotransferase enzyme, although some depletion of the PAPS (3′-phosphoadenosine-5′-phosphosulfate) pool by increasing EE_2 concentrations cannot be ruled out.

Intestinal metabolism of norgestimate

The possibility has been raised, principally on the basis of studies in female rhesus monkeys,[17] that metabolites of norgestimate (d-13-ethyl-17-acetoxy-18,19-dinor-17α-pregn-4-en-20-yn-3-oxime) and not norgestimate may be responsible for receptor binding. Sisenwine et al.[17] concluded that norgestimate appears to be a prodrug that is rapidly deacetylated to d-norgestrel-3-oxime, which is then hydrolyzed to d-norgestrel. The involvement of the intestinal mucosa in norgestimate metabolism was examined in studies with Ussing chambers.

Histologically normal terminal ileum was obtained from patients undergoing various resections, and the mucosal sheets were mounted in Ussing chambers. Radiolabeled norgestimate (0.2 μCi; 10 ng) was added to the mucosal chamber with subsequent sampling at 15, 30, 60, and 120 minutes. Conjugates were determined by hydrolysis with β-glucuronidase–sulfatase enzyme and metabolites by radiometric high-performance liquid chromatography with a mobile phase of methanol/water (70/30 v/v). Metabolite identification was by co-chromatography with authentic standards. A time course of metabolite production by intestinal mucosa is shown in Fig. 4. Note that after 2 hours of incubation, 46.3% ± 18.4% (mean ± SD) of the radioactive drug present was as unchanged norgestimate, 41.5% ± 21.6% as norgestrel oxime (deacetylated norgestimate), 4.5% ± 1.6% as norgestrel, 2.3% ± 2.3% as norgestrel acetate (loss of oxime group), and 3.6% ± 2.5% as conjugates.

These studies show very clear evidence of norgestimate metabolism, of which the principal metabolite is the deacetylated product norgestrel oxime. It seems highly probable that this will occur in vivo.

Interactions of possible clinical significance in the gut wall

Ascorbic acid. Ascorbic acid (vitamin C) forms a sulfate in the gut wall and influences the metabolism and bioavailability of other drugs that are conjugated with sulfate (e.g., salicylamide).[18] Therefore we studied the effect of concurrent administration of ascorbic acid in five women receiving long-term OC therapy.[19] The volunteers were studied in two consecutive menstrual cycles during the last week of OC administation. In random order, one of these acted as a control, and in the other week the OC was given with a 1 gm dose of ascorbic acid. Blood samples were obtained 6 and 24 hours after the OC was taken on three consecutive days and plasma was analyzed for EE_2 and levonorgestrel by radioimmunoassay.

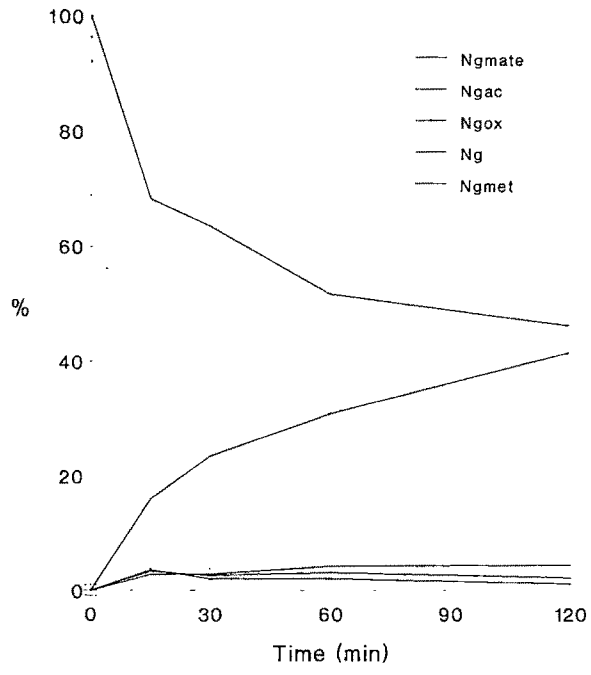


Fig. 4. Time course of metabolism of norgestimate by human mucosa (terminal ileum).

In the presence of ascorbic acid, the mean EE_2 concentrations increased at 6 hours from 49 to 57 pg · ml^{-1} (+16.3%) and at 24 hours from 21 to 31 pg · ml^{-1} (+47.6%). It was concluded that this increase was related to a decrease in gut wall metabolism caused by competition for sulfation and that high doses of vitamin C would effectively cause a low-dose OC to become a high-dose preparation.

Paracetamol. Histologically normal jejunum or terminal ileum was obtained from six patients undergoing resections, and mucosal sheets were mounted in Ussing chambers as previously described. Paracetamol, 50 μg or 1 mg, was added to the mucosal chamber (concentrations equivalent to 20 μg · ml^{-1} and 400 μg · ml^{-1} in the bathing fluid). After a period of 20 minutes, radiolabeled EE_2 (80 ng; 2 μCi) was added to the chamber, and samples were removed at intervals to 2 hours. EE_2 and conjugates were determined after differential hydrolysis and extraction.[11]

Table VI. Effect of a single dose of paracetamol (1 gm) on the AUC obtained for EE_2 for the time periods 0 to 3, 0 to 6, 0 to 11, and 0 to 24 hours

Time (hr)	*AUC (pg ml^{-1} × hr)*		*% increase*
	Control	*+ Paracetamol*	
0-3	602 ± 114	926 ± 252*	54
0-6	1010 ± 153	1417 ± 265*	40
0-11	1442 ± 166	1897 ± 320*	32
0-24	2221 ± 291	2702 ± 452*	22

From Rogers SM, Back DJ, Stevenson PJ, Grimmer SFM, Orme ML'E. Paracetamol interaction with oral contraceptive steroids: increased plasma concentrations of ethinyloestradiol. Br J Clin Pharmacol 1987;23:721-5.

Table VII. Effect of a single dose of paracetamol (1 gm) on AUC obtained for LNG, EE_2-sulfate, and EE_2-glucuronide for the time period 0 to 24 hours

	AUC_{0-24} (ng ml^{-1} × hr)	
	Control	*+ Paracetamol*
LNG	35.3 ± 12.3	39.5 ± 13.7
EE_2-sulfate	13.2 ± 4.5	7.7 ± 3.8*
EE_2-glucuronide†	8.3 ± 4.5	16.2 ± 1.8

From Rogers SM, Back DJ, Stevenson PJ, Grimmer SFM, Orme ML'E. Paracetamol interaction with oral contraceptive steroids: increased plasma concentrations of ethinyloestradiol. Br J Clin Pharmacol 1987;23:721-5.

Results are mean ± SD of six subjects except †where data are from three subjects.

*Significantly different from controls, $p < 0.05$.

The effect of paracetamol on EE_2 metabolism is shown in Table V (mucosal data only). In the presence of 50 μg of paracetamol, there was a significant decrease in total conjugates, which resulted from a decrease in formation of EE_2-sulfates. With the increase in paracetamol to 1 mg, unconjugated steroid was increased from a mean value of 56.6% (control) to 88.1%, total conjugates decreased from 35.3% to 10%, and EE_2-sulfates decreased from 33.3% to 6.8%.

These observations indicated that competition for sulfation occurred when both drugs were presented to the intestinal mucosa. Rogers et al.[11] suggested that a decrease in the PAPS (3′-phosphoadenosine-5′-phosphosulfate) pool was responsible for the decrease in steroid conjugation.

This in vitro study was followed up by examining the effect of a conventional single dose of paracetamol (1 gm) on the plasma concentrations of EE_2 in the 24-hour period after ingestion of an OC (Table VI). The study was performed in six healthy volunteers in two consecutive cycles. Plasma EE_2, EE_2-sulfate (after hydrolysis), and levonorgestrel were measured by radioimmunoassay.

The area under the plasma concentration/time curve (AUC_{0-24}) of EE_2 was significantly increased by 22% after paracetamol administration (control 2221 ± 291; after paracetamol, 2702 ± 452 pg · ml^{-1} × hour; mean ± SD; $p < 0.05$; Table VII). Plasma concentrations of LNG were unaltered by concurrent paracetamol administration.

It was concluded that the increase in plasma EE_2 concentrations resulted from a reduction in sulfation of the steroid, mainly in the gut wall, and that this interaction could be of clinical significance in women on OCs who take paracetamol regularly.

In conclusion, the studies described herein have highlighted the importance of gut wall metabolism in the overall disposition of some contraceptive steroids. EE_2 is primarily sulfated, desogestrel undergoes oxidative metabolism, whereas norgestimate principally undergoes hydrolysis with removal of the acetate group.

One of the main concerns in OC administration is to ensure that plasma concentrations are (1) adequate to achieve the contraceptive effect and (2) no higher than necessary so as to predispose to toxicity. The interactions occurring in the gut wall that have been reported (i.e., ascorbic acid and paracetamol) fall into the second category in that plasma EE_2 concentrations are raised as a result of competition for sulfation. It is also important to know if in disease states of the gastrointestinal tract (e.g., celiac disease and Crohn disease), there is any modification of metabolism. We have recently found that EE_2 sulfation was reduced by more than 60% in patients with celiac disease compared with control subjects. After a period of gluten-free treatment, metabolic activity was restored.[21] Also in vivo the bioavailability of EE_2 is significantly greater in untreated patients with celiac disease compared with healthy control subjects.

REFERENCES

1. Lehtinen A, Nurmikko V, Hartiala K. Duodenal glucuronide synthesis I. Identification of estradiol glucuronide as a conjugation product of estradiol by the rat duodenal mucosa. Qualitative studies. Acta Chem Scand 1958; 12:1585-8.

2. Lehtinen A, Hartiala K, Nurmikko V. Duodenal glucuronide synthesis. II. Identification of estradiol glucuronide as a conjugation product of estradiol by the rat duodenal mucosa. Quantitative studies. Acta Chem Scand 1958; 12:1589-91.
3. Smith FR, Tapley DT, Ross JE. Glucuronide formation in the transport of estradiol by rat intestine in vitro. Biochem Biophys Acta 1963;69:68-73.
4. Diczfalusy E, Franksson C, Martinsen B. Oestrogen conjugation by the human intestinal tract. Acta Endocrinol 1961;38:59-72.
5. Diczfalusy E, Franksson C, Lisboa BP, Martinsen B. Formation of oestrone glucosiduronate by the human intestinal tract. Acta Endocrinol 1962;40:537-51.
6. Adlercreutz H, Martin F, Jarpenpaa P, Fotsis T. Steroid absorption and enterophepatic recycling. Contraception 1979;20:201-23.
7. Back DJ, Breckenridge AM, Crawford FE, et al. An investigation of the pharmacokinetics of ethynylestradiol in women using radioimmunossay. Contraception 1979;20: 263-73.
8. Back DJ, Breckenridge AM, MacIver M, et al. The gut wall metabolism of ethinyloestradiol and its contribution to the pre-systemic metabolism of ethinyloestradiol in humans. Br J Clin Pharmacol 1982;13:325-30.
9. Back DJ, Bates M, Breckenridge AM, et al. Drug metabolism by gastrointestinal mucosa: clinical aspects. In: Prescott LF, Nimmo WS, eds. Drug Absorption. Australia: Adis Press 1980:80-7.
10. Back DJ, Bates M, Breckenridge AM, et al. The in vitro metabolism of ethinyloestradiol, mestranol and levonorgestrel by human jejunal mucosa. Br J Clin Pharmacol 1981;11:275-8.
11. Rogers SM, Back DJ, Orme ML'E. Intestinal metabolism of ethinyloestradiol and paracetamol in vitro: studies using Ussing chambers. Br J Clin Pharmacol 1987;23:727-34.
12. Maggs JL, Grabowski PS, Rose ME, Park BK. The biotransformation of 17α-ethynyl[^{3}H]estradiol in the rat. Irreversible binding and biliary metabolites. Xenobiotica 1982;12:657-68.
13. Viinikka L, Ylikorkala O, Munni S, et al. Biological effects of a new and potent progestogen. A clinical study. Acta Endocrinol 1976;83:429-38.
14. Back DJ, Grimmer SFM, Shenoy N, Orme ML'E. Plasma concentrations of 3-keto desogestrel after oral administration of desogestrel and intravenous administration of 3-keto desogestrel. Contraception 1987;35:619-26.
15. Madden S, Back DJ, Martin CA, Orme ML'E. Metabolism of the contraceptive steroid desogestrel by the intestinal mucosa. Br J Clin Pharmacol 1989;27:295-9.
16. Hartmann F, Greunke D, Craig JC, Bissell DM. Chlorpromazine metabolism in extracts of liver and small intestine from guinea pig and from man. Drug Metab Disp 1983;11:244-8.
17. Sisenwine SF, Lim AL, Kimmel HB, Ruelius HW. The conversion of d-norgestrel-3-oxime-17-acetate to d-norgestrel in female rhesus monkeys. Contraception. 1977;15:25-37.
18. Houston JB, Levy G. Modification of drug biotransformation by vitamin C in man. Nature 1975;255:78-9.
19. Back DJ, Breckendridge AM, MacIver M, Orme ML'E, Purba HS, Rowe PH. The interaction of ethinyloestradiol with ascorbic acid in man. Br Med J 1981;282:1516.
20. Rogers SM, Back DJ, Stevenson PJ, Grimmer SFM, Orme ML'E. Paracetamol interaction with oral contraceptive steroids: increased plasma concentrations of ethinyloestradiol. Br J Clin Pharmacol 1987;23:721-6.
21. Grimmer SFM, Back DJ, Orme ML'E, Tjia JF, Gilmore IT, Ellis A. In vitro conjugation of ethinyloestradiol by coeliac mucosa and its bioavailability in coeliac patients. Br J Clin Pharmacol 1986;22:217-18.

Factors affecting the enterohepatic circulation of oral contraceptive steroids

Michael L'E. Orme, MD, and David J. Back, PhD
Liverpool, England

Oral contraceptive steroids may undergo enterohepatic circulation, but it is relevant for only estrogens, because these compounds can be directly conjugated in the liver. Animal studies show convincing evidence of the importance of the enterohepatic circulation, but studies in humans are much less convincing. The importance of the route and the rate of metabolism of ethinyl estradiol are reviewed. Some antibiotics have been reported anecdotally to reduce the efficacy of oral contraceptive steroids, but controlled studies have not confirmed this observation. Although gut flora are altered by oral antibotics, the blood levels of ethinyl estradiol are not reduced, and one antibiotic at least (cotrimoxazole) enhances the activity of ethinyl estradiol. (Am J Obstet Gynecol 1990;163:2146-52.)

Key words: Enterohepatic circulation, contraceptive steroids, ethinyl estradiol, antibiotics, drug metabolism

Oral contraceptive steroids have now been in widespread use throughout the world for at least 25 years. It is currently estimated that they are used on a regular basis by 55 to 60 million women. However, scientific knowledge about steroids is still deficient in many areas, particularly about how they are handled by the body. Since the development of specific and sensitive radioimmunoassays,[1-3] we are now aware of the kinetic properties of most of the contraceptive steroids.[4] The commonly used estrogen, ethinyl estradiol (EE_2), is well absorbed from the gastrointestinal tract, but undergoes extensive, although variable, presystemic (or first-pass) elimination.[2, 3, 5] The other commonly used estrogen, mestranol, is essentially a prodrug and is metabolized by O-demethylation to EE_2 after oral absorption.[6] It appears that mestranol itself has no estrogenic activity.

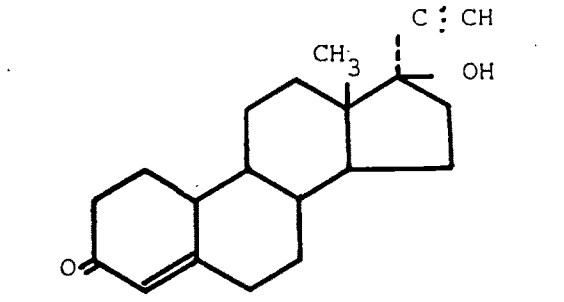
The major progestogens are all well absorbed from the gastrointestinal tract. The chemical structures of norethisterone, a "first-generation" progestogen, and EE_2, are shown in Fig. 1. There are some variations between the progestogens, but in general, first-pass metabolism is less important for the older progestogens, although norethisterone has a first-pass effect of 30% to 40%.[7] Desogestrel is metabolized extensively during the absorption process (probably mainly in the liver) to 3-ketodesogestrel, which is the active progestogen.[8] The main initial metabolic step for the progestogens is A ring reduction, which may occur at either the 3 or 5 position, and two stereoisomers of each are possible, which are designated α or β. Thus 3α, 5α; 3α, 5β; 3β, 5α; and 3β, 5β tetrahydro derivatives of norethisterone and levonorgestrel have been identified.[9, 10] In humans the evidence suggests very little direct conjugation of the hydroxyl group at the 17 position for both norethisterone[11] and levonorgestrel.[12] Thus any conjugates of norethisterone or other progestogens are most unlikely to be conjugates of the active parent drug; instead, they will be conjugates of the inactive reduced metabolites of the progestogen. Thus biliary excretion of progestogen conjugates will only be of the inactive metabolites.

From the Department of Pharmacology and Therapeutics, University of Liverpool.
Reprint requests: Michael Orme, MD, Department of Pharmacology and Therapeutics, New Medical School, Ashton Street, Liverpool, L69 3BX, United Kingdom.
6/0/23182

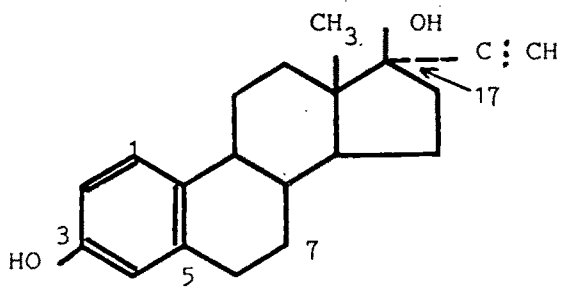
Enterohepatic recirculation of EE_2

The enterohepatic circulation of any drug involves initial conjugation, usually with glucuronic acid, in the liver to form a compound with a molecular weight in excess of 400. This is then eliminated in the bile and then goes to the gastrointestinal tract. The second part of the enterohepatic circulation involves some process that regenerates the parent (active) drug, and this is then reabsorbed into the body. Conjugates are usually regarded as being too hydrophilic to be absorbed intact. Although other mechanisms are possible, the usual process involved is a hydrolytic step performed by the gut bacteria, which in most humans are present primarily in the colon.

The enterohepatic recirculation of EE_2 is illustrated in Fig. 2. EE_2 can be conjugated with both a sulfate or a glucuronide moiety. Sulfation occurs primarily in the small intestinal mucosa,[13, 14] whereas conjugation with



NORETHISTERONE



ETHINYLOESTRADIOL

Fig. 1. The chemical structures of norethisterone and EE_2.

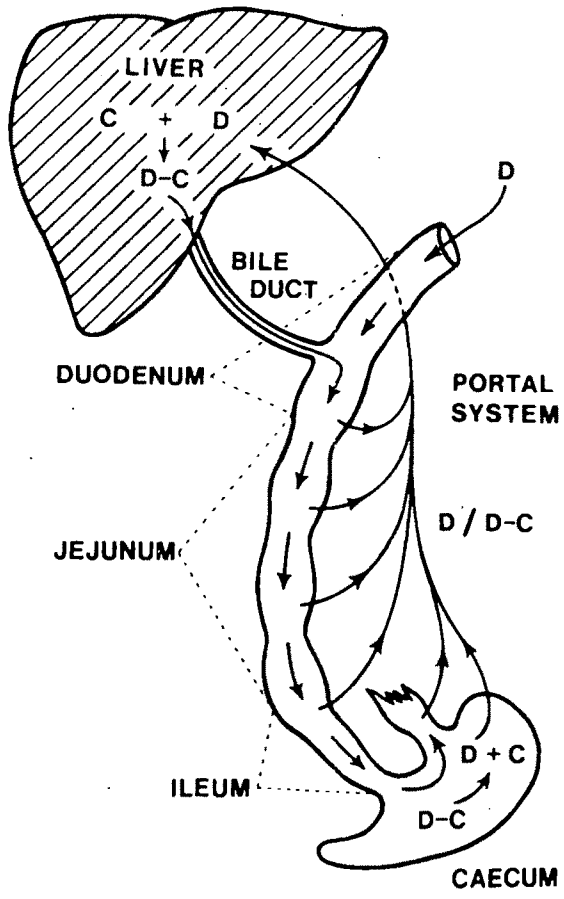


Fig. 2. Schematic representation of the enterohepatic circulation of an orally administered drug *(D)*, which is conjugated with glucuronic acid or sulfate *(C)*, to form the drug conjugate *(D-C)*, which in this example is the conjugate of EE_2.

glucuronic acid is mainly a hepatic affair.[15, 16] Conjugation of EE_2 used to be thought of as occurring primarily at the 3 position, but it is clear that glucuronidation at least can occur at the 17 position.[17, 18] These conjugates are excreted in the bile[19] and thus reach the colon. It is possible for the conjugates to reach the gut in other ways. Recent studies by Back et al.[20] with isolated human gut mucosa pieces (from which the muscularis externa is removed) mounted in Ussing chambers[21] have shown that small amounts of EE_2 conjugates can diffuse back out of the mucosa into the lumen of the gut.[20] However, this is a slow process and its relevance in vivo is not clear. It is unlikely to contribute substantially to the conjugates that are found in the gut. Once the conjugates of EE_2 reach the colon, they may be hydrolyzed by the bacteria present there to liberate unchanged EE_2, which can then be absorted into the body. This EE_2 then passes up the portal venous system to the liver for the whole process to begin again.

It is very difficult in humans to find out how important the enterohepatic recirculation of EE_2 is in practical terms. In animals there is little doubt that such a recirculation contributes substantially to blood levels of EE_2,[22] as can be shown by cutting the bile duct of one rat, catheterizing that duct, and infusing the bile into a second rat. It is, however, clearly not possible to perform this type of study in humans and even close approximations (e.g., T tube drainage) only collects a fraction of the bile produced. Fig. 3 shows a mean plasma concentration versus time profile for EE_2 compared with norethisterone in a group of five subjects. The curve for norethisterone is relatively uncomplicated and easily explicable on a two-compartment open kinetic model. The curve for EE_2 is marked by an increase in the plasma concentration at 12 to 14 hours after administration, and this is usually attributed to the enterohepatic recirculation. However, the standard error bars show that there is considerably greater interindividual variation in the kinetics of EE_2 compared with norethisterone.

Factors affecting the enterohepatic circulation of EE_2

Interindividual variation in metabolism

Rate. Examination of Fig. 3 shows that there is clearly a marked interindividual variation in the rate of metabolism of EE_2, and some of this may affect glucuron-

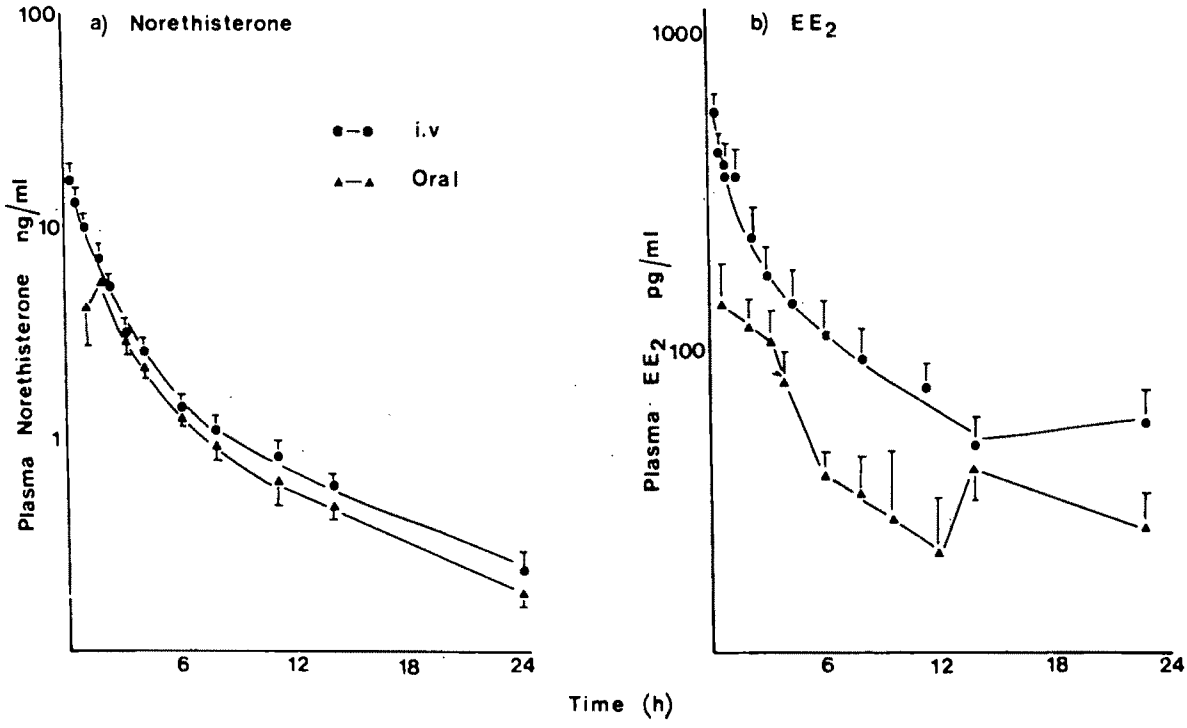


Fig. 3. Plasma concentrations of norethisterone *(a)* and EE_2 *(b)* in women after intravenous or oral administration of preparations containing norethisterone (1 mg) and EE_2 (50 μg). Each curve is the mean ± SEM of five women. (From Back DJ, Breckenridge AM, Crawford FE, et al. Drug-drug interactions: studies with oral contraceptive steroids. In: Bowers JZ, Velo GP, eds. Drug assessment criteria and methods. Amsterdam: Elsevier Science Publishers, 1979:185-206, by permission of Elsevier Science Publishers.)

idation and sulfation. In vitro studies with human gut mucosa show a variation in the sulfation of EE_2 between persons,[14] and it is likely that this also applies to glucuronidation. Drugs that are enzyme inducers are known to enhance the glucuronidation pathway as has been shown for the glucuronidation of bilirubin[23] and oxazepam.[24] Thus the known effects of enzyme inducers such as rifampicin[25] or phenobarbitone[26] in EE_2 metabolism are likely to involve both glucuronidation and hydroxylation. It is not known if enzyme inhibitors affect the glucuronidation of EE_2 in humans; however, drugs such as primaquine, which inhibit the oxidation of antipyrine in humans,[27] have no effect on the metabolism of EE_2.[28]

Route. The routes of metabolism of EE_2 are shown in Fig. 4. In most humans the main route of metabolism is by hydroxylation at the 2 position, but hydroxylation at the 4, 6, and 16 positions also occurs.[29, 30] Bolt et al.[29] showed that on average 29% of ingested EE_2 is hydroxylated at the 2 position, but in some persons this may be as high as 64%. Thus direct conjugation of EE_2 with glucuronic acid or sulfate is probably a relatively minor pathway of metabolism in most women. However, this situation may change if a person is unable to 2-hydroxylate EE_2. Maggs et al.,[31] in a study of eight patients whose bile was collected by an endoscope after a dose of ^3H-EE_2, found one woman who was unable to hydroxylate EE_2 and thus whose excretion of direct conjugates of EE_2 was increased. Genetic polymorphisms in drug metabolism are well established, and it is known that a proportion of persons are poor hydroxylators of drugs such as debrisoquine.[32] About 10% of whites belong to the poor metabolizer phenotype[33] which is inherited as an autosomal-recessive trait. The one patient in the study of Maggs et al.[31] who was unable to 2-hydroxylate EE_2 was tested for the ability to metabolize sparteine, which is metabolized by the same cytochrome P-450 as debrisoquine ($P\text{-}450_{DB}$)[34, 35] However, she was able to metabolize sparteine extensively and thus her inability to hydroxylate EE_2 was not because of a deficiency of $P450_{DB}$. Further studies are required to find out how common in the population is the inability to hydroxylate EE_2 at the 2 position. It is clear, however, that persons who are unable to perform this hydroxylation step are more likely to excrete EE_2 glucuronides and sulfate in the bile and thus have a more extensive enterohepatic circulation of EE_2.

Gastrointestinal and liver disease. Several disease processes may potentially affect the enterohepatic recirculation of EE_2, but they are unlikely to be a problem in clinical practice. Thus patients with liver disease, in whom the conjugation step may be impaired, are un-

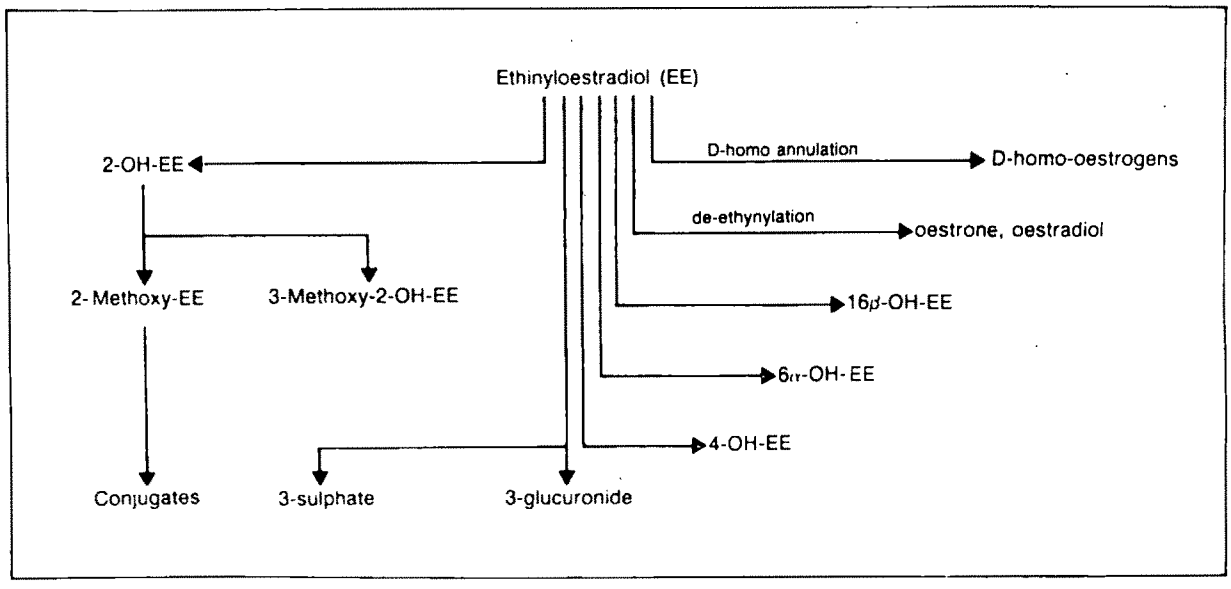


Fig. 4. Pathways of metabolism of EE_2. Metabolites may exist free or conjugated with sulfate or glucuronide. (From Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1988;8:95-136, by permission of Adis Press.)

likely to be given contraceptive steroids in the first place. Similarly, patients who have had bile duct surgery and in whom the flow of bile may be impaired will be most unlikely to be given oral contraceptive steroids.

However, in some gastrointestinal diseases patients who suffer from them are commonly given oral contraceptive steroids. The subject of gastrointestinal disease and its effect on the kinetics of contraceptive steroids are covered in another article in this supplement, and we will consider here only the disease processes that are likely to affect the enterohepatic recirculation.

In patients with active celiac disease, the ability of the gastrointestinal mucosa to conjugate EE_2 with sulfate is impaired.[36] Thus the bioavailability of EE_2 is actually increased in a disease that in general causes a malabsorptive state. However, there is no good evidence that the enterohepatic recirculation of EE_2 is reduced, and in any case after 6 months of treatment with a gluten-free diet, normal sulfate conjugation is restored to the gut mucosa.

The breakdown of the EE_2 conjugate is normally performed by gastrointestinal microorganisms, and disease processes that disturb them may alter the enterohepatic circulation of EE_2. In cystic fibrosis, in which microorganisms may be found throughout the small intestine, the kinetics of EE_2 are not different from normal, with no obvious major alteration in the enterohepatic recirculation.[37] In patients who have had an ileostomy (e.g., for ulcerative colitis), the colon is usually removed and thus the normal colonic microflora will be absent, because the ileum is usually relatively free of microorganisms. In patients who have had an ileostomy, the absorption of EE_2 is unimpaired, but there is no secondary peak of EE_2 levels at 12 to 14 hours, indicating, as expected, no (or very little) enterohepatic circulation of EE_2.[38] However, a rough comparison between the area under the plasma concentration versus time curve (AUC) for EE_2 over a 24-hour period in these patients compared with the AUC in normal volunteers given an identical dose of EE_2 reveals no significant difference between the values. Although not a controlled observation, this suggests that the contribution of the enterohepatic circulation to the overall kinetics of EE_2 is a small one in most patients. It would be interesting to compare the kinetics of EE_2 in patients before and after total colectomy, but such studies would be difficult to perform.

Changes in gut flora. As previously mentioned, gastrointestinal microorganisms are normally confined to the colon and rectum except in disease processes in which the rest of the gut may then contain bacteria. It is clear that the colon contains a very large number of bacteria and that the nature of these bacteria may vary from time to time.[39] Their nature probably depends on a variety of factors, but particularly by the food we eat and the water that we drink. Thus our gastrointestinal microorganisms may change subtly as the diet changes. More dramatic changes in gut flora, which are often accompanied by diarrhea, may be associated with travel to new destinations, where different foods and water sources may produce more profound changes in the gut flora. The hydrolysis of EE_2 conjugates is not performed by all bacteria in the gut but seems to be particulary performed by the *Clostridia* species. The work

Table I. Hydrolysis of estrone sulfates by isolated human fecal microorganisms[40]

Microorganism	*% hydrolysis*
Bacteroides	2.9
Staphylococci	6.1
Lactose-fermenting *Coliforms*	5.0
Nonlactose-fermenting *Coliforms*	3.8
Clostridia A	72.5
Clostridia B	0
Hemolytic streptococci	0.7

Further identification of *Clostrida* subtypes was not possible.

Table II. Percentage hydrolysis of estrone sulfate by human fecal microorganisms before and after preteatment with ampicillin (500 mg, four times daily, for 1½ days)[40]

Sample	*Control*	*After ampicillin*
1	95.0	1.8
2	92.6	5.4
3	90.3	8.2
4	90.1	7.5
Mean ± SEM	92.0 ± 1.0	5.7 ± 1.2*

*$p < 0.001$.

of Chapman[40] (Table I) illustrates the amount of hydrolysis of EE_2 conjugates performed by various gastrointestinal microorganisms.

Effect of antibiotics on gut flora. Antibiotics may interfere with the enterohepatic recirculation of EE_2 by killing off the *Clostridia* that are responsible for the hydrolytic process. Thus any conjugates of EE_2 would now be lost in the feces, because they are too hydrophilic to be absorbed, and potentially the plasma concentration of EE_2 would fall. Sporadic reports appeared in the literature of women who have become pregnant while taking antibiotics alone with their oral contraceptive steroids.[41-43] The number of these reports is fairly small, and some authorities have stated that the interaction is of no clinical importance.[44] However, most data sheets for the oral contraceptive steroids warn that alternative contraceptive precautions should be taken when antibiotics are taken at the same time as oral contraceptive steroids. The Committee of Safety of Medicines in the United Kingdom received 63 reports of failure of contraception in women receiving antibiotics along with their oral contraceptive steroids from 1973 to 1984.[45] The antibiotics implicated included ampicillin, penicillin, tetracyclines, and cotrimoxazole. Since the yellow card system of reporting adverse effects to the Committee on Safety of Medicines represents a considerable degree of underreporting, it is possible that this interaction may be of clinical importance, but which view is correct?

In animal studies the enterohepatic recirculation of EE_2 is extensive and is clearly diminished by treatment with antibiotics such as ampicillin or neomycin.[22] The degree of reduction of the enterohepatic recirculation can be correlated with the degree of suppression of the gut microflora. However, as already stated, it is difficult to duplicate these studies in humans. Controlled clinical studies have generally failed to show any systematic interaction between antibiotics and oral contraceptive steroids. Thus ampicillin[46-48] had no effect on the kinetics of EE_2 in women, even though the ability of the human fecal microorganisms to hydrolyze steroid conjugates is dramatically reduced by treatment (Table II).[40] Similarly, tetracycline had no clinical effect in practice.[49] In a study with cotrimoxazole there was actually a significant increase in the plasma level of EE_2 accompanied by a further suppression of follicle-stimulating hormone levels.[50] This was attributed to an inhibition of the hydroxylation of EE_2 by the sulfonamide component of cotrimoxazole. There was no evidence that the enterohepatic circulation of EE_2 had been impaired. Although these studies show no systematic interaction between antibiotics and oral contraceptive steroids scientific studies have involved no more than 50 persons, and it is possible that certain persons may be at risk. We suggest that the interaction may involve those women who are unable to hydroxylate EE_2 at the 2 position (and thus excrete larger amounts of EE_2 conjugates) and whose gut bacteria are particularly susceptible to being killed by the antibiotic used. The prevalence of this interaction is hard to guess since more work is required, but it may be close to the natural failure rate of the combined oral contraceptive steroids (0.3 to 0.5 per 100 women-years of use). One objection to some of the studies of interactions between oral contraceptive steroids and antibiotics is that bacteria in the gut develop resistance to antibiotics such as ampicillin fairly rapidly. If the antibiotic interaction is looked for after 2 weeks of antibiotic therapy, it may be missed because resistance to ampicillin develops over 5 to 6 days.[51] However, several of the aforementioned studies[46, 50] looked at the interaction during a 7-day course of antibiotics starting 3 days after the start of the antibiotic therapy at a time when resistance would be unlikely to have developed, and still no interaction was detected.

In conclusion, there is limited evidence that the enterohepatic circulation of steroids exists in humans, at least for EE_2. Some of the factors that affect that enterohepatic circulation are understood, but much more information is required before we can fully evaluate the role of the enterohepatic circulation of EE_2 and the factors that affect it.

REFERENCES

1. Warren RJ, Fotherby K. Radioimmunoassay of ethynylestradiol. J Endocrinol 1974;63:30P-1P.
2. Back DJ, Breckenridge AM, Crawford FE, et al. An investigation of the pharmacokinetics of ethynylestradiol in women using radioimmunoassay. Contraception 1979;20: 263-73.
3. Back DJ, Bates M, Breckenridge AM, et al. The pharmacokinetics of levonorgestrel and ethynylestradiol in women—studies with Ovran and Ovranette. Contraception 1981;23:229-39.
4. Orme MLE, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1983;8:95-136.
5. Back DJ, Breckenridge AM, Maclver M, et al. The gut wall metabolism of ethynylestradiol and its contribution to the pre-systemic metabolism of ethynylestradiol in humans. Br J Clin Pharmacol 1982;13:325-30.
6. Bolt HM, Bolt WH. Pharmacokinetics of mestranol in man in relation to its oestrogenic activity. Eur J Clin Pharmacol 1974;17:295-305.
7. Back DJ, Breckenridge AM, Crawford FE, et al. Pharmacokinetics of norethindrone in women. (2) Single dose pharmacokinetics. Clin Pharmacol Ther 1978;24: 448-53.
8. Hasenack HG, Bosch AMG, Käär K. Serum levels of 3-keto desogestrel after oral administration of desogestrel and 3 keto desogestrel. Contraception 1986;33:591-6.
9. Braselton WE, Lin TJ, Mills TM, Ellegood JO, Mahesh VB. Identification and measurement by gas chromatography mass-spectrometry of norethindrone and metabolites in human urine and blood. J Steroid Biochem 1977;8:9-18.
10. Sisenwine SF, Kimmel HB, Liu AL, Ruelius HW. Urinary metabolites of D L-norgestrel in women. Acta Endocrinol 1973;73:91-104.
11. Braselton WE, Lin TJ, Ellegood JO, Mills TM, Mahesh VB. Accumulation of norethindrone and individual metabolites in human plasma during short- and long-term administration of a contraceptive dosage. Am J Obstet Gynecol 1979;133:154-60.
12. Sisenwine SF, Kimmel HB, Liu AL, Ruelius HW. The presence of dl, d- and l-norgestrel and their metabolites in the plasma of women. Contraception 1975;12:339-53.
13. Back DJ, Bates M, Breckenridge AM, et al. Drug metabolism by gastrointestinal mucosa-clinical aspects. In: Prescott L, Nimmo W, eds. Drug absorption. Sydney, Australia: Adis Press, 1980:80-7.
14. Back DJ, Bates M, Breckenridge AM, et al. The in vitro metabolism of ethynylestradiol, mestranol and levonorgestrel by human jejunal mucosa. Br J Clin Pharmacol 1981;11:275-8.
15. Helton ED, Williams MC, Goldzieher JW. Human urinary and liver conjugates of 17α-ethynyloestradiol. Steroids 1976;27:851-67.
16. Helton ED, Simmons R, Meltz ML, Goldzieher JW. Variability in conjugates of ethynylestradiol produced by in vitro incubation of human liver tissue. Contraception 1977;16:257-260.
17. Helton ED, Hadd HE, Williams MC, Rao PN, Goldzieher JW. Synthesis of 17β-D-glucuopyranosiduronic acid of 17α-ethynylestradiol. J Steroid Biochem 1978;9:237-8.
18. Sahlberg B-L, Axelson M, Collins DJ, Sjovall J. Analysis of isomeric ethynylestradiol glucuronides in urine. J Chromatogr 1981;217:453-61.
19. Cargill DI, Steinetz BG, Gosnell E, et al. Fate of ingested radio labelled ethynylestradiol and its 3-cyclopentyl ether in patients with bile fistulas. J Clin Endocrinol Metab 1969;29:1051-61.
20. Rogers SM, Back DJ, Orme ML'E. Intestinal metabolism of ethynylestradiol and paracetamol in vitro: studies using Ussing chambers. Br J Clin Pharmacol 1987;23:727-34.
21. Ussing HH, Zehran U. Active transport of sodium as a source of electric current in the short circuited isolated frog skin. Acta Physiol Scand 1951;23:110-27.
22. Back DJ, Breckenridge AM, Crawford FE, et al. Reduction of the enterohepatic circulation of norethisterone by antibiotics in the rat. Correlation with changes in the gut flora. J Steroid Biochem 1980;13:95-100.
23. Orme MLE, Davies L, Breckenridge AM. Increased glucuronidation of bilirubin in man and rat by administration of antipyrine (phenazone). Clin Sci Molec Med 1974; 46:511-8.
24. Scott AK, Khir ASM, Steele WH, Hawksworth GM, Petrie JC. Oxazepam pharmacokinetics in patients with epilepsy treated long-term with phenytoin alone or in combination with phenobarbitone. Br J Clin Pharmacol 1983;16: 441-4.
25. Back DJ, Breckenridge AM, Crawford FE, et al. The effect of rifampicin on the pharmacokinetics of ethynylestradiol in women. Contraception 1980;21:135-43.
26. Back DJ, Bates M, Bowden A, et al. The interaction of phenobarbital and other anticonvulsants with oral contraceptive steroid therapy. Contraception 1980;22:495-503.
27. Back DJ, Purba HS, Park BK, Ward SA, Orme ML'E. Effect of chloroquine and primaquine on antipyrine metabolism. Br J Clin Pharmacol 1984;16:497-502.
28. Back DJ, Breckenridge AM, Grimmer SFM, Orme ML'E, Purba HS. Pharmacokinetics of oral contraceptive steroids following the administration of the antimalarial drugs primaquine and chloroquine. Contraception 1984;30:289-95.
29. Bolt WH, Kappus H, Bolt HM. Ring A oxidation of 17α-ethynylestradiol in man. Horm Metab Res 1974;6:432.
30. Williams MC, Helton ED, Goldzieher JW. The urinary metabolites of 17α-ethynylestradiol 9α-11ε-^{3}H in women. Chromatographic profiling and identification of ethynyl and non ethynyl compounds. Steroids 1975;25:229-45.
31. Maggs JL, Grimmer SFM, Orme MLE, Breckenridge AM, Park BK, Gilmore IT. The biliary and urinary metabolites of ^{3}H-17α-ethynylestradiol in women. Xenobiotica 1983; 13:421-31.
32. Maghoub A, Idle JR, Dring LG, Lancaster R, Smith RL. Polymorphic hydroxylation of debrisoquine in man. Lancet 1977;ii:584-6.
33. Steiner E, Iselius L, Alvan G, Lindsten J, Sjoqvist F. A family study of genetic and environmental factors determining polymorphic hydroxylation of debrisoquine. Clin Pharmacol Ther 1985;38:394-401.
34. Davies DS, Kahn GC, Murray S, Brodie MJ, Boobis AR. Evidence for an enzymatic defect in the 4-hydroxylation of debrisoquine by human liver. Br J Clin Pharmacol 1981;11:89-91.
35. Eichelbaum M, Defective oxidation of drugs. Pharmacokinetic and therapeutic implications. Clin Pharmacokinet 1972;7:1-22.
36. Grimmer SFM, Back DJ, Orme ML'E, Tjia J, Gilmore I, Ellis A. In vitro conjugation of ethynylestradiol by coeliac mucosa and its bioavailability in coeliac patients. Br J Clin Pharmacol 1983;22:217P-8P.
37. Stead RJ, Grimmer SFM, Back DJ, Orme ML'E, Hodson ME, Batten JC. The pharmacokinetics of the contraceptive steroids in patients with cystic fibrosis. Thorax 1987;42:59-64.
38. Grimmer SFM, Back DJ, Orme ML'E, Cowie A, Gilmore I, Tjia J. The bioavailability of ethynylestradiol and levonorgestrel in patients with an ileostomy. Contraception 1983;33:51-9.
39. Scheline R. Drug metabolism by the gastrointestinal microflora. In: Gram TE, ed. Extrahepatic metabolism of drugs and other foreign compounds. Lancaster, England: MTP Press, 1980:551-80.
40. Chapman CR. Absorption and metabolism of steroid prodrugs. PhD thesis, University of Liverpool, 1981.
41. Bacon JF, Shenfield GM. Pregnancy attributable to inter-

action between tetracycline and oral contraceptives. Br Med J 1980;1:293.
42. Dossetor J. Drug interactions with oral contraceptives. Br Med J 1975;4:467-8.
43. Hempel Von E, Bohm W, Carol W, Klinger W. Meditamentose enzyminduktion und hormonale kontrazeption. Z fur Gynakol 1979;95:1451-7.
44. Stockley IH. Drug interactions: an appraisal of the current situation. Trends Pharmacol Sci 1979;1:6.
45. Back DJ, Grimmer SFM, Orme ML'E, Proudlove C, Mann RD, Breckenridge AM. Evaluation of Committee on Safety of Medicines Yellow Card reports on oral contraceptive-drug interactions with anticonvulsants and antibiotics. Br J Clin Pharmacol 1988;25:527-32.
46. Back DJ, Breckenridge AM, Maclver M, et al. The effects of ampicillin on oral contraceptive steroids in women. Br J Clin Pharmacol 1982;14:43-8.
47. Friedman CI, Huneke AL, Kim MH, Powell J. The effect of ampicillin on oral contraceptive effectiveness. Obstet Gynecol 1980;55:33-7.
48. Joshi JV, Joshi UM, Sankhali GM, et al. A study of interaction of low dose combination oral contraceptive with ampicillin and metronidazole. Contraception 1980;22: 643-52.
49. Orme ML'E, Back DJ. Interactions between oral contraceptive steroids and broad spectrum antibiotics. Clin Exp Dermatol 1986;11:327-31.
50. Grimmer SFM, Allen WL, Back DJ, Breckenridge AM, Orme ML'E, Tjia J. The effect of cotrimoxazole on oral contraceptive steroids in women. Contraception 1983;28: 53-9.
51. Leigh DA, Reeves DS, Simmons K, Thomas AL, Wilkinson PJ. Talampicillin: a new derivative of ampicillin. Br Med J 1976;2:1378-80.

SESSION III. HEPATIC METABOLISM OF ORAL CONTRACEPTIVE STEROIDS

Chairman: M. Orme

Interactions with oral contraceptives

Kenneth Fotherby, PhD
London, England

The interaction of a range of different factors with the pharmacologic activity of oral contraceptives is reviewed. Pharmacokinetic interactions with oral contraceptives may occur (1) during absorption and extrahepatic circulation, (2) by interfering with protein binding, and (3) during hepatic metabolism. The hepatic mixed function oxidase system, which is mainly responsible for the metabolism of oral contraceptives, is affected by several different factors and is easily induced. Nutrition affects the activity of many drugs, but information regarding oral contraceptives is meager. Both pharmacokinetic and pharmacodynamic interactions, which may be synergistic or antagonistic, between the estrogen and gestagen components of oral contraceptives, are important, but there is no correlation between the rate of metabolism of the two components. Evidence suggests that some anticonvulsant, antibiotic, and antibacterial drugs may reduce the efficacy of oral contraceptives. Instances of interactions of other therapeutic agents are reported infrequently. The incidence of serious interactions is low and does not appear to have been reduced with low-dose oral contraceptives, probably because of large intersubject variability in the pharmacokinetics of oral contraceptives. (Am J Obstet Gynecol 1990;163:2153-9.)

Key words: Drug interactions, oral contraceptives, pharmacokinetics, absorption, protein binding, metabolism

Considering (1) the wide variety of drugs available, (2) the large proportion of the population consuming both prescribed and over-the-counter drugs, (3) that nutritional factors may affect drug action, and (4) that many drugs have similar metabolic pathways, it is perhaps surprising that drug interactions are not more common than they appear to be. This article is concerned only with the effects of other agents on the biologic activity of contraceptive steroids, and will not examine the changes in drug activity that may be encountered in women using oral contraceptives (OCs). As far as contraceptive steroids are concerned, a clinically important decrease in their efficacy because of interaction with other drugs appears to occur in a very small minority of women (<5%), and the possible reasons why the incidence is so low are considered later. This article examines only the possible loci in the metabolism of contraceptive steroids in which other drugs and nutrients might interact. A detailed listing of all the drugs likely to interact with the contraceptive steroids will not be provided, since many excellent accounts of this area are available.[1-4]

The interaction of drugs and nutrients with OCs may interfere with their pharmacokinetic behavior, pharmacodynamic actions, or both. In addition, with combined OCs the interaction of the estrogen and gestagen components is of great importance.

From the Department of Steroid Biochemistry, Royal Postgraduate Medical School, Hammersmith Hospital.
Reprints are not available.
6/0/23262

Pharmacokinetic interactions

The three main areas in which pharmacokinetic interactions may occur between contraceptive steroids and other drugs are (1) absorption, (2) serum protein binding, and (3) hepatic metabolism.

Absorption. There are two mechanisms by which drugs may interact with the absorption of contraceptive steroids: by affecting the absorptive process itself or by affecting the intestinal flora.

Absorption of steroids may be impaired when the rate of which they pass through the gastrointestinal tract is markedly increased because of increased intestinal motility; for example, in subjects either using laxatives or with severe or prolonged diarrhea. Ethinyl estradiol (EE) and some gestagens, such as norethisterone (NET), undergo extensive (40% to 60%) first-pass metabolism, much of which occurs in the intestinal wall. Absorption appears to occur mainly in the upper part of the intestine, since the bioavailability of EE and levonorgestrel (LNG) is not decreased by ileostomy or after jejunoileal bypass. Phase 1 metabolism of steroids can be demonstrated with intestinal tissue in vitro, and intestinal tissue contains cytochrome P-450, but under normal in vitro conditions, phase 1 metabolism by the intestine appears to be unimportant. However, the ex-

tent to which such metabolism is increased after the administration of enzyme-inducing drugs has not been investigated.

Specific effects on EE, which undergoes sulfation in the intestinal mucosa, may occur with certain drugs. Since the amount of available sulfate appears to be limited, administration in high doses of other drugs that are sulfated during absorption, such as vitamin C and paracetamol, leads to competition, less EE is sulfated, and its bioavailability is improved. Conversely, when the competing drug is stopped, more sulfation of EE occurs and its bioavailability is reduced.

Contraceptive steroids also undergo an enterohepatic circulation, and this is particularly important for EE. The steroids conjugated in the liver are secreted into the intestine by way of bile and are hydrolyzed by the gut flora. If these are inhibited by the administration of antibiotics, hydrolysis of the conjugates is markedly reduced, which leads to reduced reabsorption of the steroids and an increase in the fecal excretion. The effects on the gestagens are clinically less important, since they are rapidly metabolized to biologically inactive products by the liver, mainly by reduction, and to a lesser extent by hydroxylation before conjugation and secretion in the bile. Metabolism of EE in the liver involves hydroxylation at various positions, and in subjects who are poor hydroxylators, more EE is available for conjugation and the extrahepatic circulation. In these subjects interference with the extrahepatic circulation produced by the administration of antibiotics may lead to markedly lower serum EE concentrations.

Although the effect of some antibiotics on the gut flora provides one mechanism by which the bioavailability of contraceptive steroids, particularly EE, may be decreased, whether this is the major factor involved in the few clinically important interactions that occur remains to be conclusively demonstrated.

Binding to serum proteins. The contraceptive steroids are highly bound (>95%) in blood and thus are susceptible to factors that may produce only slight modifications of the binding. Thus an insignificant 2% decrease in the amount bound could lead to a significant 50% increase in the unbound fraction on which the biologic activity of the steroids depends. EE binds only to albumin, and although interaction between drugs in respect to albumin has been demonstrated, no interaction between contraceptive steroids and this protein appears to have been reported. However, the gestagens bind both to albumin and sex hormone–binding globulin (SHBG), the extent and tightness of the binding to SHBG depending on their structure, and there is a complex equilibrium between the different fractions. Serum SHBG levels can be increased by the administration of estrogens and several other drugs. The increase in SHBG may lead to an increase in the SHBG bound fraction, mainly at the expense of the albumin bound, from which dissociation occurs readily. Because contraceptive steroids are administered on a continuing basis, their serum concentrations will increase until a steady state is reached and a fresh equilibrium is formed among the different fractions; at this point the proportion unbound may be decreased, but the concentration of unbound may be unchanged. Therefore although there may be marked changes in the relative binding, these changes are unlikely to influence the efficacy of contraceptive steroids. These interactions between estrogen, gestagens, and SHBG are considered in more detail in a recent article.[5]

Hepatic metabolism. The most important locus of drug interaction appears to be the mixed function oxidase system of the liver endoplasmic reticulum. This system occurs in several other tissues, but for most drugs the liver is the major site. The enzyme system has a low specificity, performing a number of different metabolic transformations, all of which involve cytochrome P-450, cytochrome C reductase, a flavoprotein, and reduced nicotinamide adenine dinucleotide phosphate, and its activity is stimulated by many drugs. As a result, not only is the administered drug metabolized more quickly, with a decrease in its blood concentration and biologic activity, but so are several other drugs that may be administered concurrently. There are a number of distinct P-450s,[5] each of which may be under separate genetic control, and this may account for some of the interindividual variability in the rate of drug metabolism. The low specificity of the enzyme system as a whole enables it to metabolize a large number of different drugs and endogenous compounds, including the gonadal and adrenocortical steroids. Each of the distinct cytochrome P-450s may exert its main activity toward groups of substrates, with a variable amount of overlap between groups, and this accounts for the competitive inhibition that can occur among different substrates.

In addition to genetic factors, the rate of drug metabolism is influenced by a large number of environmental factors, diet, alcohol use, smoking, medication, and pathologic conditions, and all these various factors may affect the mixed function oxidase activity of the liver. Despite the low or overlapping specificity of the mixed function oxidase system, there is often marked selectively, both positional and stereochemically, in the metabolism of the substrate. With the steroids, which can be oxidized at several different sites in the molecule, the resulting hydroxylations occur to a variable extent and they can be inhibited differently by various factors. Again this suggests that multiple forms of cytochrome P-450 are present with different specifications. The relative activities of the different cytochrome P-450s ap-

pear to vary among subjects, and if so, it is important in accounting for much of the interindividual variation in drug metabolism.

The increase in the activity of the mixed function oxidase system produced by the administration of an inducing drug leads to an increase in the rate of metabolism of any contraceptive steroid the subject may use, so that the half-life of elimination of the steroid, and consequently its serum concentration and biologic effect, are decreased. Conversely, the administration of contraceptive steroids usually has an inhibitory effect on the metabolism of other drugs, which leads to an increase in their pharmacologic activity.[3]

The mechanism of this inhibitory effect in humans is not entirely clear. In animals, mainly the rat, the administration of steroids containing the ethinyl group, which encompasses most of the contraceptive steroids currently used, appears to inactivate cytochrome P-450, with a consequent decrease in the drug-metabolizing capacity of the liver. It was first demonstrated in humans in 1969 that EE may be metabolized by way of oxidation of the ethinyl group, which results in the formation of D-homosteroids and small amounts of estrone and estradiol. The oxidation of the ethinyl group is a complex reaction involving the production of numerous reactive intermediates. It appears that one or more of these metabolites interacts with, and leads to the destruction of, the heme group of cytochrome P-450, with a consequent loss of its drug-metabolizing activity both toward the steroid and other administered drugs metabolized by the mixed function oxidase system.[7-9] As a result, the pharmacologic effect of such drugs is increased. There is evidence of the in vitro formation of D-homosteroids from ethinyl steroids in the rabbit and monkey, in addition to humans.[10] Several other chemical structures, in addition to the ethinyl group, may have a similar destructive effect on cytochrome P-450.[11] An increased excretion of porphyrin precursors and coproporphyrin was reported to occur in women taking OCs,[12] which could indicate an increased turnover of cytochrome P-450 heme.

One reactive intermediate in the oxidation of the ethinyl group is an epoxide, and these compounds may also be formed by oxidation at the carbon 4-5 double bond of the steroids.[13] Interestingly, the epoxides of norethisterone (NET) and levonorgestrel (LNG) may show differences in their biologic activities.[14] However, these epoxides do not appear to be implicated in heme destruction, so that the effect produced by oxidation of the ethinyl group must be from some other reactive intermediate. Many questions still remain to be answered in respect to the inhibition of cytochrome P-450. Destruction appears to apply only to the heme part, which could readily be resynthesized; the apoprotein appears to be unaffected. So far heme destruction has been demonstrated only in the rat and in in vitro studies; whether it occurs and to what extent in humans remains to be determined. In women metabolism of the ethinyl group is only a minor pathway for EE and probably even less so for NET and LNG. Destruction of cytochrome P-450 by metabolites of the ethinyl steroids provides one mechanism by which contraceptive steroids may impair the metabolism of coadministered drugs. Whether the concentration of the reactive intermediates attains a level sufficient to impair the cytochrome P-450 system with short-term administration of contraceptive steroids seems unlikely, although drug interactions may occur under these conditions. However, with long-term use of steroidal contraception, the situation may be different. A decreased concentration of cytochrome P-450 was found in liver microsomes from monkeys that had received contraception steroids for at least 10 years,[15] although the factors responsible need to be identified. Even if heme destruction by contraceptive steroids with a consequent reduction in the drug-metabolizing capacity of the liver occurs in humans, it does not rule out the possibility that other mechanisms may exist and may be more important for the steroidal inhibition of drug metabolism. Even small doses of gestagens administered without estrogen may inhibit drug metabolism.[16] Conversely, Mills et al.[17] reported that the clearance of NET increased in women treated with 2.5 mg of NET daily. Any of these mechanisms provides a means by which contraceptive steroids are able to inhibit their metabolism, with a consequent increase over time in the pharmacologic effects.

Nutritional effects

In considering nutritional effects, it is necessary to distinguish between effects related to the quantity of the diet and those related to its quality. The former will depend on whether caloric intake in relation to energy expenditure is very low, leading to a low body mass index (BMI) and in extreme cases to anorexia nervosa, or very high, leading a high BMI and obesity. The quality will depend on the composition of a diet that is satisfactory in relation to caloric requirements. The influence of diet and nutrition on the clinical pharmacokinetics of drugs in general has been reviewed.[18]

Food intake affects absorption and bioavailability of several drugs[19, 20] but little information is available regarding contraceptive steroids.

Absorption of some lipid-soluble drugs is increased by a high-fat diet, but no investigations have been performed to determine whether this also applies to lipid-soluble contraceptive steroids. The pharmacokinetics of many drugs may be altered in obese subjects, particularly lipid-soluble drugs such as steroids, for which the apparent volume of distribution may be greatly

increased. However, in a preliminary comparison of two gestagens in healthy subjects, medroxyprogesterone acetate and NET enanthate given by intramuscular injection so that the effects of intestinal absorption were eliminated, differences in the pharmacokinetics between obese (BMI > 28) and thin (BMI < 20) subjects were minimal.[21] The elimination half-life of NET was significantly lower (mean, 5.9 hours) in a group of Indian women in a low socioeconomic group with poor nutritional status compared with that (13.4 hours) of well-nourished women in a high socioeconomic group.[22] Similar findings have also been reported for LNG.[23] The extent to which these findings can be ascribed to low serum protein levels in the former group remains to be determined. In a preliminary study of estradiol, and presumably the same situation holds for EE, 2-hydroxylation was increased and 16α-hydroxylation was decreased in anorexic subjects, whereas the reverse was observed in obese subjects.[24]

High-protein diets appear to stimulate the activity of the hepatic drug–oxidizing system, with a consequent increased rate of metabolism of many drugs. The 2-hydrozylation of estradiol is enhanced but apparently without any concomitant change in 16α-hydrozylation,[18] and the activity of the hepatic 5α-reductase is decreased. High-carbohydrate diets decrease 2-hydrozylation. A low-fat diet was also reported to increase 2-hydroxylation of estradiol and to decrease 16α-hydroxylation,[25] although this diet was also probably a high-protein one. Diet also has a marked influence on endogenously secreted androgens in humans,[26] and by analogy might be expected to affect the metabolism of gestagens. Certain specific nutrients, such as charcoal-broiled beef and cruciferous vegetables, stimulate the hepatic mixed function oxidase system.

Whether metabolism of contraceptive steroids differs between vegetarians and nonvegetarians is still controversial. Vegetarian diets tend to contain less fat and more fiber than nonvegetarian diets, and these differences appear to influence the metabolism of endogenous estrogens, which leads to a decrease in plasma estradiol concentrations.[27] This decrease might be partly caused by a decrease in the enterohepatic circulation. However, there were no significant differences in the pharmacokinetics of NET between lactovegetarian and nonvegetarian women.[28] One determinant of whether there are differences in drug metabolism between vegetarians and nonvegetarians appears to be the amount of protein in the diet.

In many of the situations discussed above, changes occur in serum SHBG concentrations, and while these changes will not affect EE, which does not bind to SHBG, they will affect the unbound biologically active fraction of the gestagens. Serum SHBG shows an inverse relationship to body weight, being low in obese subjects and increased in anorexic subjects. SHBG may also be increased in subjects consuming a low-fat diet and in vegetarians. In the latter this may be partly from absorption of phytoestrogens in the fiber-rich diet. The level of free fatty acids in serum affects the binding of endogenous sex hormones to serum proteins and increases their biologically active unbound fractions.[29, 30] Therefore it seems likely that contraceptive steroids, which elevate serum lipid levels, will be similarly affected.

Estrogen-gestagen interactions

Interaction between the estrogen and gestagen components of combined OCs may occur both at the pharmacodynamic and pharmacokinetic levels. In respect to pharmacodynamics, the interaction may be synergistic or antagonistic, whereas in some pharacodynamic responses the two components may act independently, for example, the effect of estrogen in elevating the levels of cortisol-binding globulin or triglyceride concentrations in serum. For four monophasic combined OCs containing approximately the same dose of EE but with different gestagens, the increase in triglyceride concentrations was approximately the same. Examples of synergism between the two components would be the transformation of a proliferative into a secretory endometrium and the inhibition of ovulation. With the low doses of EE and gestagen in currently used OCs, ovulation is inhibited in almost all treatment cycles when the two components are coadministered, whereas neither component alone in the same dose would produce consistent inhibition.

For most pharmacodynamic responses, the interaction is an antagonistic one, for example, the effects on high-density lipoprotein cholesterol, in which the increase produced by EE can be suppressed to a variable extent by gestagens depending on their structure and dose. The antagonistic action can clearly be seen in the effect on serum SHBG concentrations, for whereas the level of high-density lipoprotein cholesterol can be subject to many factors that may cause large variability in its measurement, serum levels of SHBG can be measured with precision and accuracy by simple methodology. As shown in Fig. 1, the SHBG concentration depends on the relative doses of estrogen (EE) and gestagen (NET) administered.

Evidence is accumulating for pharmacokinetic interactions between estrogen and gestagen. One point of interaction is SHBG. As indicated above, serum SHBG level is increased by estrogens, and this may affect the pharmacokinetics of those gestagens such as LNG or gestodene (GES), which bind strongly to SHBG.[5] However, whether any clinical effects result from these changes is not clear.

In a study in which women were sampled at intervals during 1 year of treatment with two OCs,[31] serum EE concentrations were consistently higher in women receiving the OC containing GES than in those receiving desogestrel, although the dose of EE was the same in both formulations. This finding suggests that the gestagen influenced the metabolism of EE. Since EE does not bind to SHBG, changes in the serum concentration of this protein cannot account for the observed effect, and it seems likely that in women taking the GES formulation, the metabolic clearance of EE was reduced to a greater extent than in those taking EE and desogestrel. The mechanism of this effect remains to be determined but considering the effect of ethinyl steroids in decreasing the activity of cytochrome P-450 (see above), one interpretation would be that GES has more pronounced inhibitory action on cytochrome P-450 than does desogestrel. In another study,[32] serum EE concentrations were consistently higher as expected in women who had taken an OC for 6 months than in a control group, but the clearance rate of EE was on average 35% lower in OC users than in control subjects, suggesting a decrease in the metabolizing capacity of the OC group. In this study metabolism of concomitantly administered theophylline was also decreased, suggesting the reduction in metabolic capacity was a generalized effect; again it seems reasonable to postulate that the findings are the result of an inhibitory effect of ethinyl steroids on cytochrome P-450 activity.

The increased levels of LNG in women using OCs containing EE and LNG compared with those receiving the same dose of LNG without EE has been attributed to the EE-induced increase in SHBG and the binding of LNG to SHBG, with a consequent reduction in its clearance and rate of metabolism. However, as demonstrated recently,[33] this conclusion is erroneous. Thus the slower rate of metabolism of LNG on continued dosing must be the result of other factors. Another study[34] found that the clearance rate of LNG decreased and the half-life of elimination increased in long-term users of a OC containing LNG and EE. Therefore both of these findings suggest an increase in the resistance of LNG to metabolism on continued dosing, and again this may be the result of a decrease in the activity of the cytochrome P-450 system.

In a study of the pharmacokinetics of NET and EE in a large group of women taking an OC containing these contraceptive steroids,[35] no correlation was found between the rate of metabolism of the two steroids as measured by their half-lives of elimination. Although these two closely related steroids are metabolized by the hepatic drug–metabolizing system, this lack of correlation suggests that the metabolic reactions were catalyzed by different enzymes or different variants of cytochrome P-450.

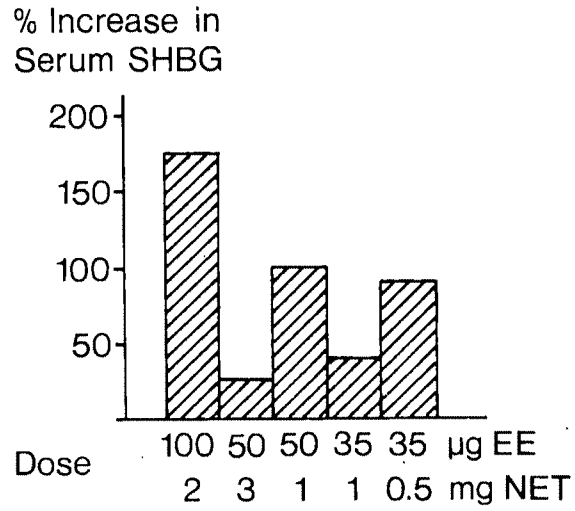


Fig. 1. Changes in serum SHBG concentrations in relation to dose of estrogen (EE) and gestagen (NET). (Reproduced with permission from Fotherby K. Ann NY Acad Acad Sci 1988; 538:313-20.)

Smoking

Smoking will increase the metabolism of some drugs, whereas others are unaffected. Similarly, the rate of metabolism of some endogenous steroids, such as cortisol and progesterone are reported to be increased, whereas that of others, such as estrogens and testoterone are unchanged or decreased. The cause of the variability is unclear. Smoking leads to the production of a large number of chemicals, particularly the polycyclic aromatic hydrocarbons, which are stimulators of the hepatic drug–metabolizing system. Evidence of an effect on this system is supported by the finding[36] that 2-hydroxylation of estradiol was greater in smokers (43%) than in nonsmokers (25%). In a preliminary study[37] the rate of metabolism of EE and LNG was not affected by smoking, and variable results were obtained by Kanarkowski et al.[34] Clearly, definitive studies of the effect of smoking on the metabolism of contraceptive steroids are required.

Conclusions

It seems clear[38, 39] that the two main areas of drug interaction that affect the pharmacokinetics and efficacy of the OCs are first the effect of some, but not all, anticonvulsant drugs, antibiotics, and antibacterial agents on the hepatic drug–metabolizing system, which leads to an increased rate of metabolism of contraceptive steroids, and second, the effect of some, but again not all, antibiotics and antibacterial drugs on the absorption and enterohepatic circulation of contraceptive steroids; this latter effect will mainly reduce the serum concentration of EE, and the gestagen will be little affected. Effects of other therapeutic agents on contraceptive steroids are reported much more rarely, and in

these circumstances it is difficult to know whether the reduced efficacy of the OC is caused by the concomitantly administered drug or by some other event, such as the subject forgetting to take one or more pills, particularly near the beginning or end of the treatment period, or to the infrequent but nevertheless adequately documented occurrence of ovarian activity, leading in a few instances to breakthrough ovulation and the risk of pregnancy.[40, 41] Even when there is the possibility of an interaction, only in a small proportion of women (probably well under 5%, although no proper epidemiologic studies have been performed to determine the incidence of drug interactions with the OCs) will the interaction occur to such an extent that there is a decrease in the efficacy of the OC, thereby leading to the risk of pregnancy. It might be expected that serious interactions would be more common now that most OCs used contain low doses of contraceptive steroids. However, there appears to be little evidence to support this contention.[39] One reason for this may be the marked interindividual variability in the pharmacokinetics of contraceptive steroids.[42] One might postulate that serious interactions would occur only in those women who are fast metabolizers and therefore have low serum concentrations of contraceptive steroids and in those women whose liver enzyme systems are particularly susceptible to induction.

More information is required regarding the mechanisms by which contraceptive steroids produce their inhibitory effect on many coadministered drugs, and particularly since it is important with regard to long-term use of OCs, the way in which contraceptive steroids may inhibit their metabolism.

REFERENCES

1. Park BK, Breckenridge AM. Clinical implications of enzyme induction. Clin Pharmacokinet 1981;6:1-24.
2. Stockley IH. Drug interactions. London: Blackwell Scientific, 1981.
3. D'Arcy PF. Drug interactions with oral contraceptives. Drug Intell Clin Pharmacol 1986;20:353-61.
4. Griffin JP, D'Arcy PF, Speirs CJ. Manual of adverse drug reactions. 4th ed. London: Wright, 1988.
5. Fotherby K. Potency and pharmacokinetics of gestagens. Contraception 1990;41:533-50.
6. Lu AYH, West SB. Multiplicity of mammalian microsomal-cytochromes P450. Pharmacol Rev 1980;31:277-92.
7. White INH. Metabolic activation of acetylenic substituents. Biochem J 1978;174:853-61.
8. Blakey DC, White INH. Destruction of cytochrome P450 by contraceptive steroids. Biochem Pharmacol 1986;35:1561-7.
9. Guengerich FP. Oxidation of 17α-ethynylestradiol by human liver cytochrome P450. Mol Pharmacol 1988;33:500-8.
10. Fotherby K. Species differences in metabolism of contraceptive steroids. In: Gregoire AT, Blye RT, eds. Contraceptive steroids. New York: Plenum Press, 1986:113.
11. Ortiz de Montellano PR, Correia MA. Suicidal destruction of cytochrome P450. Ann Rev Pharmacol 1983;23:481-503.
12. Koskelo P, Eisalo A, Toivonen I. Urinary excreation of porphyrin precursors in oral contraceptive users. Br Med J 1966;1:652-4.
13. White INH, Eberhard UM. Decreased liver cytochrome P450 in rates caused by norethindrone or ethynyloestradiol. Biochem J 1977;166:57-64.
14. Peter H, Jung R, Bolt HM, Oesch F. Norethisterone and levonorgestrel oxides. J Steroid Biochem 1981;14:83-90.
15. Schmid SE, Au WYW, Hill DE, Kadlubar FF, Shikker W. Cytochrome P450 dependent oxidation of the ethynyl group of synthetic steroids. Drug Metab Dispos 1983;11:531-6.
16. Field B, Lu C, Hepner GW. Inhibition of hepatic drug metabolism by norethindrone. Clin Pharmacol Ther 1979;25:196-8.
17. Mills TM, Lin TJ, Hemandy S, Greenblatt RB, Ellegood JO, Mahesh VB. MCR and urinary excretion of norethindrone. Am J Obstet Gynecol 1974;120:764-72.
18. Anderson KE. Influences of diet and nutrition on clinical pharmacokinetics. Clin Pharmacokinet 1988;14:325-46.
19. Welling PG. Influence of food and diet on drug absorption. J Pharmacokinet Biochem 1977;5:291-334.
20. Melander A, McLean A. Influence of food intake on presystemic clearance of drugs. Clin Pharamcokinet 1983;8:286-96.
21. Fotherby K, Koetsawang S. Metabolism of injectable contraceptive steroids in obese and thin women. Contraception 1982;26:51-8.
22. Prasad KVS, Rao BSN, Sivakumar B, Prema K. Pharmacokinetics of norethindrone in Indian women. Contraception 1979;20:77-90.
23. Madharan NK, Sirakumar B, Prema K, Rao BSN. Pharmacokinetics of levonorgestrel in Indian women. Contraception 1979;20:303-12.
24. Fishman J, Bradlow HL. Effect of malnutrition of the metabolism of sex hormones. Clin Pharmacol Ther 1977;22:721-8.
25. Longcope C, Gorbach S, Goldin S, et al. Effect of a low fat diet on oestrogen metabolism. J Clin Endocrinol Metab 1987;64:1246-9.
26. Belanger A, Locung A, Noel C, et al. Influence of diet on plasma steroid and SHBG levels. J Steroid Biochem 1989;32:829-33.
27. Aldercruetz H, Hockerstedt K, Bannwart C, et al. Effect of dietary components on liver metabolism of estrogens. J Steroid Biochem 1987;27:1135-44.
28. Prasad RNV, Fotherby K, Jenkins N, Ratnam SS. Single dose kinetics of norethisterone in lactovegetarians. Singapore J Obstet Gynaecol 1981;12:59-67.
29. Reed MJ, Beranek PA, Cheng RW, James VHT. Free fatty acids, a possible regular of available oestradiol fractions. J Steroid Biochem 1986;24:657-9.
30. Mooradian AD, Pamplona DM, Viosea SP, Korenman SC. Effect of free fatty acids on bioavailability of plasma testroterone. J Steroid Biochem 1988;29:369-70.
31. Jung-Hoffman C, Kuhl H. Interaction with the pharmacokinetics of ethynyloestradiol of progestogens in oral contraceptives. Contraception 1989;40:299-312.
32. Tornatore KM, Kanarkowski R, McCarthy TL, Gardner MJ, Yarchak AM, Jusko WJ. Effect of chronic OC steroids on theophylline disposition. Eur J Clin Pharmacol 1982;23:129-34.
33. Fotherby K. Pharmacokinetics of gestagens—some problems. Am J Obstet Gynecol 1990;163:323-8.
34. Kanarkowski R, Tornatore KM, Ambrosio R, Gardner MJ, Jusko WJ. Pharmacokinetics of single and multiple doses of EE and LNG. Clin Pharmacol Ther 1988;43:23-31.
35. Shi YE, He CH, Gu J, Fotherby K. Pharmacokinetics of norethisterone in humans. Contraception 1987;35:465-75.
36. Michnovicz JJ, Herschcopf RJ, Haley NJ, Bradlow HL,

Fishman J. Cigarette smoking alters hepatic estrogen metabolism. Metabolism 1989;38:537-41.
37. Crawford P, Back DJ, Orme ML, Breckenridge AM. Oral contraceptive steroid plasma concentrations in smokers and non-smokers. Br Med J 1981;282:1829-30.
38. Back DJ, Grimmer SF, Otma ML, Proudlove C, Mann RD, Breckenridge AM. Evaluation of reports on oral contraceptive—drug interactions. Br J Clin Pharmacol 1988;25:527-32.
39. Szoka PR, Edgren RA. Drug interactions with oral contraceptives. Fertil Steril 1988;49:315-85.
40. Westcombe R, Ellis R, Fotherby K. Suppression of ovulation in women using a triphasic oral contraceptive. Br J Fam Plann 1987;13:127-32.
41. Sparrow MJ. Pill method failures. NZ Med J 1987;100: 102-5.
42. Fotherby K. Variability of pharmacokinetic parameters for contraceptive steroids. J Steroid Biochem 1983;19: 817-20.
43. Fotherby K. Interactions of contraceptive steroids with steroid binding proteins and the clinical implications. Ann NY Acad Sci 1988;538:313-20.

Inhibition of oral contraceptive steroid–metabolizing enzymes by steroids and drugs

F. Peter Guengerich, PhD
Nashville, Tennessee

The major 17α-ethinyl estradiol 2-hydroxylase is humans is the hepatic enzyme cytochrome P-450 IIIA4 (P-450NF), which is known to be inducible by rifampicin or barbiturates. The literature indicates that 17β-estradiol, progesterone, and norgestrel are competitive inhibitors and that primaquine and tolbutamide are rather weak noncompetitive inhibitors. Recent experiments in this laboratory indicate that gestodene is a relatively potent mechanism-based inactivator of cytochrome P-450 IIIA4 in vitro. Inhibition requires incubation with the reduced form of nicotinamide adenine dinucleotide phosphate, is time and concentration dependent, and can be partially blocked by the presence of noninhibitory cytochrome P-450 IIIA4 substrates. The in vitro activation by gestodene provides a possible explanation for the increase in plasma estrogen levels reported in women administered gestodene along with 17α-ethinyl estradiol. (Am J Obstet Gynecol 1990;163:2159-63.)

Key words: 17α-Ethinyl estradiol, 2-hydroxylation, gestodene, inhibition, of enzymes, cytochrome P-450 IIIA4

The main estrogenic component of oral contraceptives is 17α-ethinyl estradiol, which was introduced by Innhoffen and Hohlweg.[1] Its usefulness appears to result from its slow elimination relative to 17β-estradiol. Considerable variation is seen in the rates of clearance of ethinyl estradiol among women,[2-4] and such differences cause difficulty in adjusting the drug level. In addition to the normal variation seen among persons, another problem is introduced by the induction and inhibition of enzymes involved in the metabolism of ethinyl estradiol. 2-Hydroxy ethinyl estradiol, the major product, is also estrogenic but is not retained in the body as long as the parent compound. Menstrual problems and unexpected pregnancies have been experienced in some women using rifampicin or barbituates, and the effects can be rationalized in the decreased levels of ethinyl estradiol measured both in vivo and in vitro.[5-9] Bolt et al.[8] also demonstrated that rates of hydroxylation of estradiol were increased in women after treatment with rifampicin, with the effect presumably caused by enzyme induction.

From the Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine.
Supported in part by U.S. Public Health Service grants CA44353 and ES00267.
Reprint requests: F. Peter Guengerich, PhD, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232.
6/0/22642

Identification of cytochrome P-450$_{NF}$ as ethinyl estradiol 2-hydroxylase

Human liver P-450NF was first isolated as the enzyme catalyzing the oxidation of the calcium channel blocker nifedipine.[10] In that study the enzyme was also the principal catalyst in the main pathways of testosterone and estradiol metabolism in human liver, namely, 6β- and 2-hydroxylation, respectively. Further studies in this

Table I. Substrates for human liver cytochrome P-450 IIIA enzymes (P-450$_{NF}$)

Steroids	*Drugs*	*Carcinogens*
Testosterone (6β)	Nifedipine	Aflatoxin B_1
Estradiol (2,4)	Quinidine (3, *N*)	1,6-Dinitropyrene
Progesterone (6β)	Erythromycin	*tris*(Dibromopropyl)phosphate
Cortisol (6β)	Cyclosporine	6-Aminochrysene
Ethinyl estradiol (2)	Midazolam	Benzo*(a)*pyrene-7,8-diol

See Guengerich et al.,[10-12] Waxman et al.,[31] Shimada and Guengerich,[32] Shimada et al.,[33,34] Ged et al.,[35] and Guengerich[36] for original experiments and leading references.

and other laboratories have led to the assignment of this enzyme in a variety of other reactions involving the oxidation of steroids, drugs, and carcinogens (Table I).[11]

With the demonstration of the estradiol 2-hydroxylase activity of P-450NF and the suggestive evidence for induction of the enzyme by rifampicin (and the previous studies on rifampicin–ethinyl estradiol interactions[8]), the possibility was raised that P-450NF might be the human liver ethinyl estradiol 2-hydroxylase. Indeed such was the case; specific antibodies raised against P-450NF inhibited >90% of both the nifedipine oxidase and ethinyl estradiol 2-hydroxylase activities in human liver microsomes containing either high- or low-catalytic activity.[12] Rates of ethinyl estradiol 2-hydroxylation were well correlated with both rates of nifedipine oxidation and immunochemically determined P-450NF in liver samples obtained from different persons. Furthermore, troleandomycin (which has been shown to selectively inhibit cytochrome P-450 enzymes in the P-450 IIIA family)[13] was converted to a (nitroso) metabolite that blocked both nifedipine oxidation and ethinyl estradiol 2-hydroxylation. Several lines of evidence indicate that other cytochrome P-450 enzymes do not catalyze the reaction, including P-450DB (IID6), P-450PA (IA2), P-450$_j$ (IIE1), and the enzymes in the P-450 IIC family.[12]

It should be pointed out that P-450NF (IIIA4) is a member of a complex multigene family, with at least three proteins having >80% sequence identity.[14-16] The full details of specific expression and regulation of the genes are not understood at this time, and the possibility exists that more than one of these related proteins might contribute to ethinyl estradiol 2-hydroxylation and be recognized by the antibodies. Another point is that specific forms of cytochrome P-450 involved in ethinyl estradiol 2-hydroxylation in animal models have not been identified. If the 2-hydroxylation of estradiol can be used as a guide, then the rat model may be more complex. We previously reported that in contrast to the human liver, in which P-450NF is the principal catalyst of 2-hydroxylation, at least three cytochrome P-450 enzymes (the products of three different gene families) contribute to the reaction in the rat liver.[17]

Inhibitors of ethinyl estradiol hydroxylation

Purba et al.[18] examined several drugs and steroids for their ability to inhibit ethinyl estradiol 2-hydroxylation in vitro (in human microsomes, K_m 9 μmol/L). Several steroids were found to be competitive inhibitors, including estradiol (K_i 12 μmol/L), progesterone (K_i 69 μmol/L), and norgestrel (K_i 69 μmol/L). Primaquine and tolbutamide were noncompetitive inhibitors (K_i 69 μmol/L and 81 μmol/L, respectively). Several other compounds were tested and found to be rather poor inhibitors, including chloroquine, 1-methylimidazole, cholesterol, cortisol, diethylstilbesterol, antipyrine, mephenytoin, and metronidazole. Bolt and Kassel[19] reported that the insecticide synergist, 1-naphthyl-4(5)-imidazole, was a competitive inhibitor of microsomal ethinyl estradiol 2-hydroxylation (K_i 3 μmol/L) on the rat liver. Troleandomycin has already been shown to be an inhibitor of microsomal ethinyl estradiol 2-hydroxylation in vitro in the human liver.[12] Such macrolide antibiotics are known to be effective inhibitors in vivo[13] and one would also expect erythromycin to be a selective inhibitor of ethinyl estradiol 2-hydroxylation.

Mechanism-based inactivation by acetylenic steroids

In addition to the types of inhibition previously mentioned, mechanism-based or "suicide" inactivation of cytochrome P-450 is seen with the acetylenic steroids. White and Muller-Eberhard[20] reported that treatment of rats with ethinyl estradiol or norethindrone decreased the level of cytochrome P-450, and White[21] demonstrated the formation of green pigments formed from the heme of cytochrome P-450 in rats treated with ethinyl testosterone. Subsequently, the heme adducts formed by acetylenic steroids have been interpreted in terms of mechanism-based inactivation.[22-23] Mechanism-based enzyme inactivation is of significance in that only the enzyme involved in the biotransformation of the compound becomes inactivated, and the damage is therefore targeted.[24] Many of the acetylenic steroids appear to behave as inhibitors in this way.[25]

Although the chemistry of the green *N*-alkylporphyrins resulting from heme destruction has been deduced by Ortiz de Montellano et al., Davies et al.[26] have subsequently found that during the oxidation of norethindrone by rat liver microsomes, a considerable portion of the heme destroyed becomes irreversibly attached to the cytochrome P-450 apoprotein. The 2-hydroxy ethinyl estradiol that is formed by metabolism is readily oxidized in vitro to an ortho quinone that can covalently bind to protein,[9] but the significance of this reaction is unclear.

Ethinyl estradiol has been found to be a mechanism-based inactivator of human liver microsomal cytochrome P-450 and P-450NF (in vitro). The partition ratio, or number of times that an enzyme turns over a substrate before it is inactivated,[24] was estimated at ~120.[12]

Inhibition of cytochrome P-450 by acetylenic progestogens

Since progestogens are commonly used with ethinyl estradiol in contraceptive formulations, their interactions with estrogens are of great importance.[27] Kuhl et al.[28] reported that gestodene levels rise during therapy, and they considered the possibility that the increase was due to decreased metabolism. Furthermore, Kuhl et al.[28] found that in comparison with desogestrel, gestodene had a marked effect in increasing plasma levels of ethinyl estradiol, and they also showed that the cause was decreased clearance of the drug. In addition, cortisol levels were elevated in patients treated with gestodene, which might be expected if cytochrome P-450NF, the cortisol 6β-hydroxylase, was inhibited (Table I).

A series of 17α-acetylenic steroids was examined for the ability to inactivate human liver microsomal nifedipine oxidase and ethinyl estradiol 2-hydroxylase, activities of cytochrome P-450NF (Fig. 1). All the steroids proved to be inactivators, but clearly gestodene was the most effective when tested at the single concentration of 100 μmol/L. Indeed, the extent of inhibition seen with gestodene under these conditions was more marked than that found with a 20 μmol/L concentration of the classic inhibitor troleandomycin, which reduced the activity of nifedipine oxidase to 21% and that of ethinyl estradiol 2-hydroxylase to 65% under these conditions. The effects of gestodene were examined in more detail, the inhibition pattern is classic for mechanism-based inactivators[24] in that inactivation is first order in time and the rate increases with inhibitor concentration. The data can be used to derive the parameters K_i = 46 μmol/L and $k_{inactivation}$ = 0.39 minute.$^{-1}$ The $k_{inactivation}$ value is one of the highest ever measured for a microsomal cytochrome P-450 enzyme.[29]

In other studies it was possible to demonstrate the loss of spectrally detectable cytochrome P-450 in human liver microsomes during the oxidation of gestodene. In this particular sample, which is enriched with P-450NF, the level of cytochrome P-450 was decreased to 40% and thereafter remained constant. The difference in the rates of destruction of cytochrome P-450 by gestodene and ethinyl estradiol is quite dramatic, and logarithmic plots indicate that the rate is approximately 50-fold greater in the case of gestodene.

Gestodene was effective in destroying microsomal cytochrome P-450, even at the lower concentration of 10

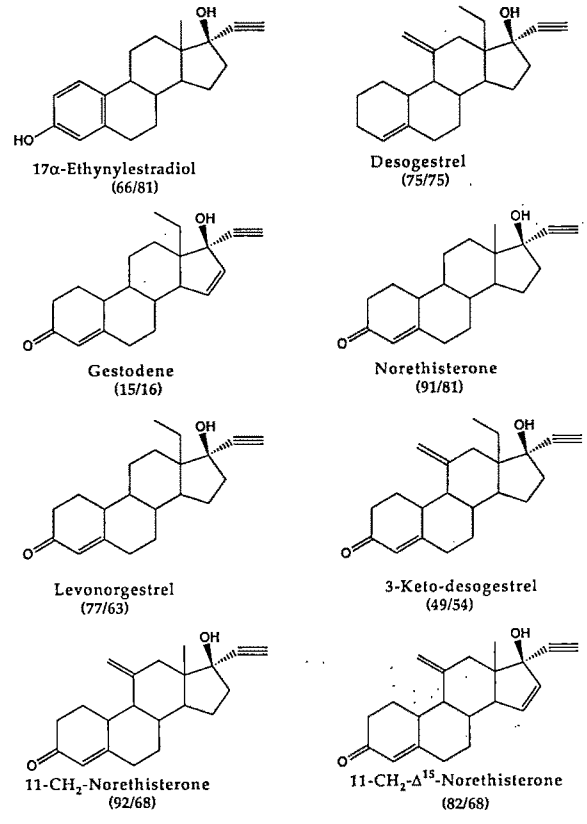


Fig. 1. Structures of synthetic 17α-ethynyl and 17β-hydroxy steroids examined as mechanism-based inactivators of human liver microsomal cytochrome P-450. Each steroid (100 μmol/L) was incubated with 250 pmol of human liver cytochrome P-450 (liver sample HL 110) and an NADPH-generating system in a reaction volume of 50 μl at 37° C for 30 minutes. At that time the reaction was diluted to a volume of 1.0 ml with the NADPH-generating system and either 200 μmol/L nifedipine or 100 μmol/L 17α-ethinyl estradiol, and these incubations proceeded for an additional 10 minutes. Rates of nifedipine oxidation and 17α-ethinyl estradiol 2-hydroxylation were determined by high-performance liquid chromotagraphy and rates of the activities relative to those measured in similar experiments without steroids are presented (as percentages of uninhibited values). The first of each pair of numbers *(in parentheses)* indicates the relative rate of nifedipine oxidation, and the second indicates the relative rate of ethinyl estradiol 2-hydroxylation. *NADPH*, Nicotinamide adenine dinucleotide phosphate.

μmol/L (57% ± 4% cytochrome P-450 remaining after 10 minutes). The destruction could be partially blocked by high concentrations of the P-450NF substrates quinidine (100 μmol/L, 76% ± 1% original cytochrome P-450 after 10 minutes) and nifedipine (200 μmol/L, 82% ± 4% original cytochrome P-450 remaining after 10 minutes). The K_m values for quinidine and nifedipine are 4 and 19 μmol/L, respectively.[10,11] Comparison of the rates of nifedipine oxidase inactivation and gestodene disappearance (at a single concentration of gestodene) yields an estimated partition ratio of 9 gestodene, which can be compared with the value of 120 for ethinyl estradiol.[12] Therefore a given dose of gestodene

would be expected to inactivate 15 times as much cytochrome P-450NF as does ethinyl estradiol.

More than 10 gestodene metabolites are known,[27] with biotransformation resulting from the formation of the 3α- and 3β-hydroxyl compounds, 4, 5-hydrogenation, hydroxylation at the 1, 6α, 11 and other unidentified positions, and homoannulation (conversion of the D ring to a cyclohexanone with release of CO_2).[30] It is somewhat surprising that a 6β-hydroxy product has not been identified, since 6β-hydroxylation is observed in the cytochrome P-450NF oxidations of the Δ^4 3-keto steroids testosterone, androstenedione, cortisol, and progesterone.

Conclusions

P-450NF (and possibly closely-related enzymes) is the major catalyst involved in the 2-hydroxylation of ethinyl estradiol, the major step in the clearance of the compound in the human liver. The enzyme appears to be induced by the administration of rifampicin, barbiturates, or certain steroids such as dexamethasone. The enzyme catalyzes the oxidation of several steroids, drugs, and carcinogens (Table I), and variations in the activity of the enzyme would alter the rates of biotransformation of all these compounds. Most of the inhibitors of the enzyme require metabolism to exert their effects. All 17α-acetylenic steroids examined are mechanism-based inactivators of P-450NF and its activities, but gestodene shows a remarkably high activity in this regard. This property of the compound can explain its ability to elevate plasma levels of ethinyl estradiol and cortisol. Examination of the effects of the different steroids tested does not permit elucidation of the structure/function relationships related to mechanism-based inactivation of P-450NF. Because the inhibition seen with gestodene is mechanism based, only those cytochrome P-450–mediated activities associated with P-450NF (Table I) would expected to be compromised in patients receiving the drug. Finally, gestodene appears to be an excellent compound to be used in the further in vitro and possibly in vivo examination of which compounds are oxidized by cytochrome P-450NF.

REFERENCES

1. Innhoffen HH, Hohlweg W. Neue per os-wirksame weibliche Keimdrüsenhormon-Derivate: 17-Äthinylöstradiol und Pregnen-in-on-3-ol-17. Naturwissenschaften 1938; 26:96.
2. de la Pena A, Chenault CB, Goldzieher JW. Radioimmunoassay of unconjugated plasma ethynylestradiol in women given a single dose of ethynylestradiol or mestranol. Steroids 1975;25:773-80.
3. Stadel BC, Sternthal PM, Schlesselman JJ, et al. Variation of ethinylestradiol blood levels among healthy women using oral contraceptives. Fertil Steril 1980;33:257-60.
4. Jung-Hoffman C, Kuhl H. Interaction with the pharmacokinetics of ethinyl estradiol of progestogens contained in oral contraceptives. Contraception 1989;40:299-312.
5. Reimers D, Ježek A. Rifampicin und andere Antituberkulotika bei gleichzeitiger oraler Kontrazeption. Prax Pneumol 1971;25:255-62.
6. Nocke-Finck L, Brewer H, Reimers D. Wirkung von Rifampicin auf den Menstruationszylkus und die Östrogenausscheidung bei Einnahme oraler Kontrazeptiva. Dtsch Med Wochenschr 1973;98:1521-3.
7. Janz D, Schmidt D. Anti-epileptic drugs and failure of oral contraceptives. Lancet 1974;1:1113.
8. Bolt HM, Kappas H, Remmer H. Studies on the metabolism of ethynylestradiol in vitro and in vivo: the significance of 2-hydroxylation and the formation of polar products. Xenobiotica 1973;3:773-85.
9. Bolt HM. Metabolism of estrogens—natural and synthetic. Pharmacol Ther 1979;4:155-81.
10. Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ. Characterization of the rat and human microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype of genetic polymorphism in oxidative drug metabolism. J Biol Chem 1986;261:5051-60.
11. Guengerich FP, Müller-Enoch D, Blair IA. Oxidation of quinidine by human liver cytochrome P-450. Mol Pharmacol 1986;30:287-95.
12. Guengerich FP. Oxidation of 17α-ethynylestradiol by human liver cytochrome P-450. Mol Pharmacol 1988;33: 500-8.
13. Pessayre DV, Descatoire V, Konstantinova-Mitcheva M, et al. Self-induction by triacetyloleandomycin of its own transformation into a metabolite forming a stable 456 nm-absorbing complex with cytochrome P-450. Biochem Pharmacol 1981;30:553-8.
14. Nebert DW, Nelson DR, Adesnik M, et al. The P450 superfamily: update on listing of all genes and recommended nomenclature of the chromosomal loci. DNA 1989;8:1-13.
15. Beaune PH, Umbenhauer DR, Bork RW, Lloyd RS, Guengerich FP. Isolation and sequence determination of a human liver cytochrome P-450 clone related to human cytochrome P-450 nifedipine oxidase. Proc Natl Acad Sci USA 1986;83:8064-8.
16. Bork RW, Muto T, Beaune PH, Srivastava PK, Lloyd RS, Guengerich FP. Characterization of mRNA species related to human liver cytochrome P-450 nifedipne oxidase and regulation of catalytic activity. J Biol Chem 1989;264: 910-9.
17. Dannan GA, Porubek DJ, Nelson SD, Waxman DJ, Guengerich FP. 17β-Estradiol 2- and 4-hydroxylation catalyzed by rat hepatic cytochrome P-450: roles of individual forms, inductive effects, developmental patterns, and alterations by gonadectomy and hormone replacement. Endocrinology 1986;118:1952-60.
18. Purba HS, Maggs JL, Orme M L'E, Back DJ, Park BK. The metabolism of 17α-ethinyloestradiol by human liver microsomes: formation of catechol and chemically reactive metabolites. Br J Clin Pharmacol 1987;23:447-53.
19. Bolt HM, Kasssel H. Effects of insecticide synergists on microsomal oxidation of estradiol and ethynylestradiol and on microsomal drug metabolism. Xenobiotica 1976; 6:33-8.
20. White INH, Muller-Eberhard U. Decreased liver cytochrome P-450 in rats caused by norethindrone or ethynyloestradiol. Biochem J 1977;166:57-64.
21. White INH. Metabolic activation of acetylenic substituents to derivatives in the rat causing the loss of hepatic cytochrome P-450 and haem. Biochem J 1978;174:853-61.
22. Ortiz de Montellano PR, Kunze KL, Yost GS, Mico BA. Self-catalyzed destruction of cytochrome P-450: covalent binding of ethynyl sterols to prosthetic heme. Proc Natl Acad Sci USA 1979;76:746-9.
23. Ortiz de Montellano PR, Kunze KL. Self-catalyzed inactivation of hepatic cytochrome P-450 by ethynyl substrates. J Biol Chem 1980;255:5578-85.
24. Silverman RB. Mechanism-based enzyme inactivation:

chemistry and enzymology. Vols 1 and 2. Boca Raton, Florida: CRC Press, 1988.
25. Ortiz de Montellano PR, Correia MA. Suicidal destruction of cytochrome P-450 during oxidative drug metabolism. Annu Rev Pharamacol Toxicol 1983;23:481-503.
26. Davies HW, Britt SG, Pohl LR. Inactivation of cytochrome P-450 by 2-isopropyl-4-pentenamide and other xenobiotics leads to heme-derived protein adducts. Chem-Biol Interact 1986;58:345-52.
27. Düsterberg B, Tack J-W, Krause W, Hümpel M. Pharmacokinetics and biotransformation of gestodene in man. In: Elstein M, ed. Gestodene: development of a new gestodene-containing low dose oral contraceptive. Carnforth: Parthenon Publishers, 1987:35-44.
28. Kuhl H, Jung-Hoffman C, Heidt F. Serum levels of 3-keto-desogestrel and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 150 μg desogestrel. Contraception 1988;38:381-90.
29. Hammons GJ, Alworth WL, Hopkins NE, Guengerich FP, Kadlubar FF. Mechanism-based inactivation of cytochrome P-450-dependent 2-naphthylamine *N*-oxidation activity of liver microsomes by 2-ethynylnaphthalene. Chem Res Roxicol 1989;2:367-374.
30. Schmid SE, Au WYW, Hill DE, Kadlubar FF, Slikker WM Jr. Cytochrome P-450-dependent oxidation of the 17α-ethynyl group of synthetic steroids: *D*-homoannulation or enzyme inactivation. Drug Metab Dispos 1983;11:531-6.
31. Waxman DJ, Attisano C, Guengerich FP. Lapenson DP. Cytochrome P-450 steroid hormone metabolism catalyzed by human liver microsomes. Arch Biochem Biophys 1988;263:424-36.
32. Shimada T, Guengerich FP. Evidence for P-450NF, the nifedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. Proc Natl Acad Sci USA 1989;86:462-5.
33. Shimada T, Iwasaki M, Martin MV, Guengerich FP. Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA1535/pSK1002. Cancer Res 1989;49:3218-28.
34. Shimada T, Martin MV, Pruess-Schwartz D, Marnett LJ, Guengerich FP. Roles of individual forms of human cytochrome P-450 enzymes in the bioactivation of benzo(*a*)pyrene, 7,8-dihydroxy-7,8-dihydrobenzo (*a*pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. Cancer Res 1989;49:6304-12.
35. Ged C, Rouillon JM, Pichard L, et al. Cortisol 6β-hydroxylase in human liver microsomes. Br J Clin Pharmacol 1989;28:373-87.
36. Guengerich FP. Biochemical characterization of human cytochrome P-450 enzymes. Annu Rev Pharmacol Toxicol 1989;29:241-64.

Formation, metabolism, and physiologic importance of catecholestrogens

Peter Ball, Prof. Dr. med,[a] and Rudolf Knuppen, Prof. Dr. Rer. Nat.[b]
Lübeck, West Germany

The metabolism of natural and synthetic estrogens is governed primarily by hydroxylations, leading to polyhydroxylated derivatives of the steroid molecule. In mammals aromatic hydroxylation is most prominent quantitatively. The 2- and 4-hydroxyestrogens (catecholestrogens) formed are secreted not only in high amounts in urine but are also present in significant quantities in different organs, such as the liver, pituitary gland, and hypothalamus. This A ring hydroxylation of primary estrogens is affected by peroxidases, tyrosinases, and unspecific monooxygenases by mechanisms still not completely understood. The activity of the aromatic hydroxylases is regulated not only with respect to the overall extent but also to the relative rate of hydroxylation at C-atoms 2 and 4. The metabolism of catecholestrogens may be divided into reversible and irreversible reactions, of which the reaction with the catechol-O-methyltransferase, and thereby the interaction with catecholamines, the conjugation, and the thioether formation are the most prominent. Low- and high-affinity binding is operative in binding to plasma proteins and receptors. Finally, irreversible binding to cellular macromolecules, such as proteins and deoxyribonucleic acid, and the oncogenic potential of natural and synthetic catecholestrogens are discussed. (Am J Obstet Gynecol 1990;163:2163-70.)

Key words: Catecholestrogens, formation, metabolism, humans

From the Klinische Endokrinologie,[a] and Biochemische Endokrinologie, Medizinische Universität zu Lübeck.[b]
Reprint requests: Dr. P. Ball, Klinische Endokrinologie, Medizinische Universität zu Lübeck, D-2400 Lübeck West Germany.
6/0/23623

Since the discovery of the "classic" estrogens—estrone (E_1), estradiol (E_2), and estriol (E_3)—some 50 years ago, intense research work has been carried out on the metabolism of these hormones that, before and

Table I. Concentrations of 2-substituted E_1 in plasma of humans (picograms per milliliter)

Subject	*2-OHE$_1$ unconjugated/conjugated*	*2-OCE$_1$ unconjugated/conjugated*
Children, 5-9 yr	20/—	60/1130
Men	45/1000	80/1320
Women		
Periovulation	65/2300	180/1180
Pregnant	100/5000	3980/5850
Children, newborn	500/3000	—/—

Table II. Excretion of 2-substituted E_1 in urine of humans (micrograms per 24 hours)

Subject	*2-OHE$_1$*	*2-OCE$_1$*	*2-Substituted/total E*
Children	1.0	2.4	1.3
Men	9.0	12	1.6
Women			
Cycling	30	20	0.7
Pregnant	700	450	—
Menopausal	5.0	9.0	1.7

in addition to conjugation, are essentially transformed by hydroxylation reactions. In mammals these hydroxylations occur not only at the aliphatic C-atoms 18, 16, 15, 14, 11, 7, and 6, but also at aromatic positions 4 and 2. This aromatic ortho-hydroxylation of phenols, also well known in the biotransformation of many nonsteroidal compounds with physiologic and pharmacologic importance, has several interesting features that stimulated intense and steadily increasing investigations into 2- and 4-hydroxyestrogens in recent years.

Because of the evident structural relationship between E_2, ethinyl estradiol (EE), and in part tyrosine, the reactions given here for the natural estrogens are, in general, the same for all phenols.

Metabolism of E_2 in vitro in rat and human liver and in vivo in humans[1, 2]

When [6,7-^{3}H]E_2 is incubated with rat liver slices for 1 hour under O_2, approximately 50% of the radioactivity remains lipophilic, 30% becomes water soluble, and 4% is irreversibly bound to protein.

In the water-soluble fraction containing the "classic" conjugates, conjugates with sulfuric and glucuronic acid, and the "newer" conjugates, mainly glutathione, the sulfate esters predominate. Within the more than 20 metabolites isolated from the lipophilic and, after hydrolysis, water-soluble fractions of rat liver, the main metabolites are 2-hydroxylated estrogens (25%), followed by their isomeric monomethyl ethers (15%), 4-hydroxyestrogens are of minor importance (8%).

Essentially the same distribution pattern of metabolites is found on incubation of human liver slices, although the turnover of the substrate is reduced.

In the same patient whose in vitro metabolism in the liver was explored, [4-^{14}C]E_2 was administered orally, and the excretion pattern of radioactive metabolites was monitored in urine. Again, 2-substituted estrogens were the main products (10%), although they did not exceed the sum of primary (E_1 + E_2 = 8%) and 16α-hydroxylated (16α-OHE$_1$ and E_3 = 6%) estrogens.

Concentrations of endogenous 2-substituted estrogens in tissue, blood, and urine

Using a monoisotope-derivative method (radioenzymatic assay) and ether extracts of the tissues, preliminary values for the concentration of 2-hydroxyestrone (2-OHE$_1$) in the female rat were 560 pg/gm for the liver, 770 pg/gm for the pituitary gland, and 150 pg/gm for hypothalamic tissue. In comparison, rat plasma contained about 520 pg/ml.[3]

With the use of newly developed radioimmunoassays for all relevant catecholestrogens, the determination of plasma concentrations became feasible. The obtained normal values in humans are given in Table I.[3, 4] Unconjugated and conjugated steroids were determined separately. The 2-OHE$_1$ concentrations were in the range of those of E_1, and the 2-OHE$_1$ 2-methyl ether (2-OCE$_1$) concentrations were even slightly higher than those of E_1 and 2-OHE$_1$ together. Determinations of the conjugated 2-OHE$_1$ and 2-OCE$_1$ concentrations resulted in 10 to 20 times higher values than those obtained for the unconjugated steroids.

Using the same radioimmunoassays, urinary excretion rates of 2-substituted and 4-substituted estrogens were monitored.[3] The results for the 2-substituted estrogens are given in Table II. Of special interest were the molar ratios of 2-substituted to 2-nonsubstituted estrogens, the so-called total estrogens. With the exception of women in the reproductive phase, the amount of 2-substituted estrogens clearly surpassed the

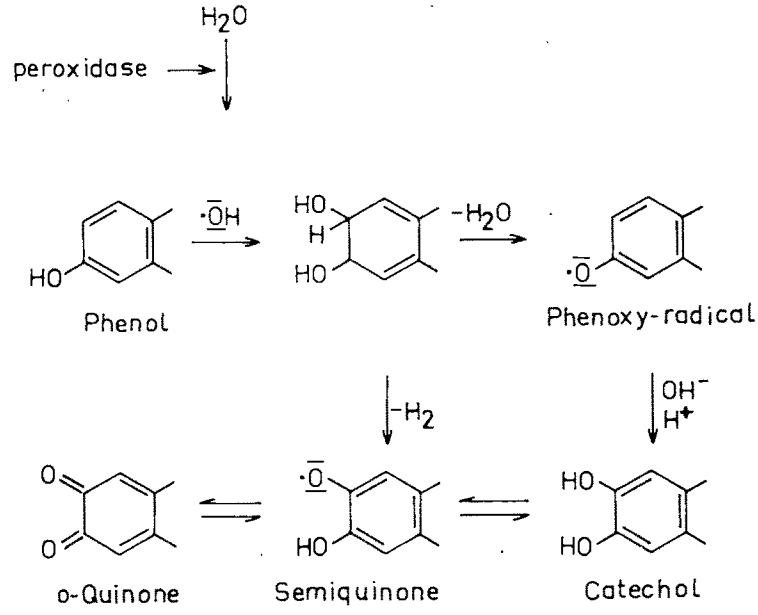


Fig. 1. Peroxidase-proposed mechanism of action. (From Kuss E. Estrogens and glutathione. In: Dolphin D, Voulson R, Avramovic O, eds. Glutathione. New York: John Wiley & Sons, 1989:512-49. Copyright© 1989 by John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

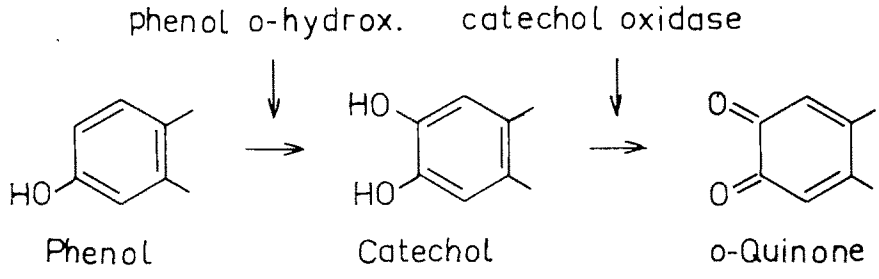


Fig. 2. Tyrosinase-catalyzed reactions.

amount of all other estrogens together. For the excretion rates of 2-OHE_1 and 4-OHE_1 during the menstrual cycle of four healthy subjects, see ref. 4.

Formation of catecholestrogens: Enzymatic hydroxylation of estrogens

Enzymatic A ring hydroxylation of primary estrogens is thought to be affected by three different hydroxylating systems: peroxidases, tyrosinases, and the "unspecific monooxygenases."

The mechanism of action of these three enzymes is still poorly understood. It is strongly interconnected with glutathione metabolism and thus thioether formation.[5] It is believed that peroxidases, through the generation of hydroxyl radicals, initially oxidize phenols to phenoxy radicals, eventually leading to dihydroxybenzenes (catechols), partly in a semiquinone or o-quinone–reactive form (Fig. 1). These quinones show a high binding to deoxyribonucleic acid and protein, a reaction that may be competitively inhibited by glutathione (GS-SG and thioether formation) and lipids (lipid peroxidation).[3] Recently, the estrogen 2-/4-hydroxylase activity of the soluble fraction of human breast cancer tissue was found to behave similarly to the peroxidase type from the rat uterus.[6]

Tyrosinases that show both phenol o-hydroxylase and catechol oxidase activity catalyze a two-electron transfer to form o-quinones (Fig. 2). The quinones have the remarkable ability to conjugate with reactive groups on the tyrosinase molecule itself and thus have the potential to inhibit the further action of the enzyme (product inhibition from the suicide type). The addition of glutathione enhances tyrosinase activity, presumably by protecting functional groups of the enzyme or reactive groups of the product, thereby inhibiting the suicide reaction by enhancing not only thioether conjugation but also the formation of protein adducts. Similar reactions were observed with EE and dopa as substrates.[5]

However, the classic estrogen 2-/4-hydroxylase seems

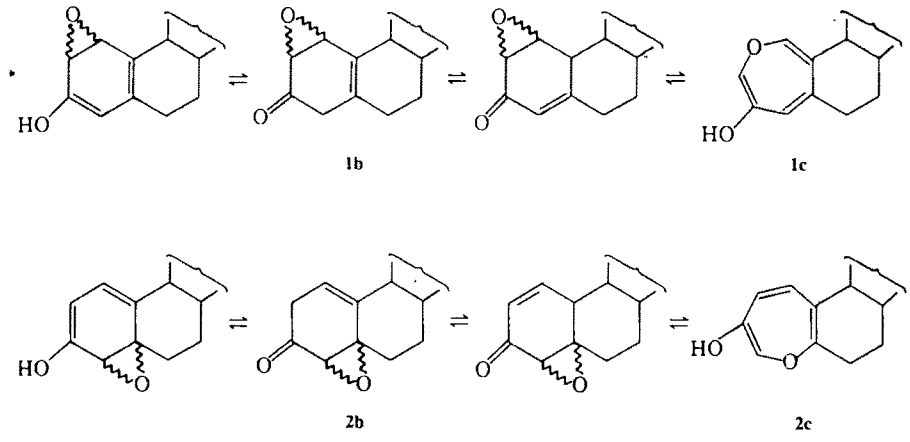


Fig. 3. Hypothetic intermediates in the biosynthesis of 2- and 4-hydroxyestrogens. (From Kuss E. Estrogens and glutathione. In: Dolphin D, Voulson R, Avramovic O, eds. Glutathione. New York: John Wiley & Sons, 1989:512-49. Copyright© 1989 by John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

to be a cytochrome P-450–dependent microsomal enzyme system. The labile 1,2- and 4,5-arenoxides (dienol epoxides) (Fig. 3), in equilibrium with the more stable keto- and oxepin tautomers, are the postulated intermediates. Arenoxides are unlikely precursors of thioethers, which need an o-quinone, or more likely a semiquinone radical, as molecular species immediately preceding their formation.[5]

Hydroxylase activity is strongly dependent on the nature of the substrate: 17α-EE is a far better substrate than are natural estrogens (EE > E_2 > E_1).

Enzymatic activity is increased by higher age and the male sex—androgens and castration[7]—and a widely varying array of substances such as phenobarbital,[5] EE,[8] and rifampicin,[9, 10] and decreased by cytochrome P-450 inhibitors,[5] 2- and 4-haloestrogens,[11] and growth hormone.[12] Finally, urinary excretion of 2-hydroxyestrogens was increased by malnutrition, hyperthyroidism, and tobacco smoking and decreased by liver cirrhosis.[3, 5] Because the C-2 and C-4 hydroxylations are strongy correlated (ratio of activity 10:1 to 20:1), the same mechanism seems to be operative in both reactions. Nevertheless, the relative C-2/C-4 reaction rates vary depending on both the substrate and the source of the enzyme.[1, 13]

Metabolism and binding of catecholestrogens

To elucidate the importance of catecholestrogens in physiology, the rate of formation is one important parameter; the metabolism and binding are others. Within the latter we must discriminate between enzymatic (nonenzymatic) metabolism of the catecholestrogens, both reversible and irreversible, and low- and high-affinity binding, with functional preservation of the catechol structure.

One of the reversible interactions is the reaction with enzymes such as the catechol-O-methyltransferase (COMT). In 1958 Axelrod and Tomchick[14] already showed that the COMT is one of the key enzymes in the metabolism of catecholamines. We purified the enzyme from the liver, characterized it, and proved Knuppen's hypothesis[15] that the enzymatic methylation of 2-hydroxyestrogens and catecholamines is accomplished by the same methyltransferase.[16] Moreover, we showed that under standard assay conditions, catecholestrogens were not only the preferred substrates (Table III)[17], but also the most potent inhibitors of the methylation of catecholamines in vitro (Fig. 4).[18-20] We suggested that the inhibition of catecholamine metabolism by the catecholestrogens might cause prolongation and potentiation of the physiologic actions of the catecholamines, especially blood pressure and gonadotropin release.[21]

As demonstrated already by Axelrod and Tomchick, COMT is an ubiquitous enzyme. Of special interest is its high activity in red blood cells,[22] which leads to the high plasma metabolic clearance rate of catecholestrogens (Table IV)[23] and falsely low catecholestrogen concentrations if the blood is handled incorrectly.[22] Catecholestrogens monomethyl ethers are prone to extensive demethylation. On incubation with rat liver slices, apparent Kms of 12 and 3 μmol/L for 2-OCE_1 and 2-OCE_2 were obtained.[17]

Catecholestrogens are metabolized by two other reversible reactions: conjugation with glucuronic and sulfuric acid on incubation of [4-^{14}C]2-OHE_2 or [4-^{14}C]E_2 with rat liver slices; the most prominent finding is the extensive conjugation of the catecholestrogens,[24] a conjugation rate that is significantly higher (58%) than that of the primary estrogen (47%).

Finally, two irreversible reactions of catecholestrogens should be mentioned. It has long been known that

Table III. Substrate specificity of COMT from human liver

Substrate	*K_m (μmol/L)*
2-OHE_2	15
2-OHE_1	20
4-OHE_1	20
Dopamine	250
Epinephrine	300
EE	10

K_m, Michaelis constant.

Table IV. Plasma metabolic clearance rates of catechol estrogens in humans

CE	*MCR (l/day)*
2-OHE_1	28.000
2-OHE_2	12.000
4-OHE_1	15.000
2-OCE_1	2.000
E_2	400

CE, Catechol estrogens; *MCR*, metabolic clearance rate.

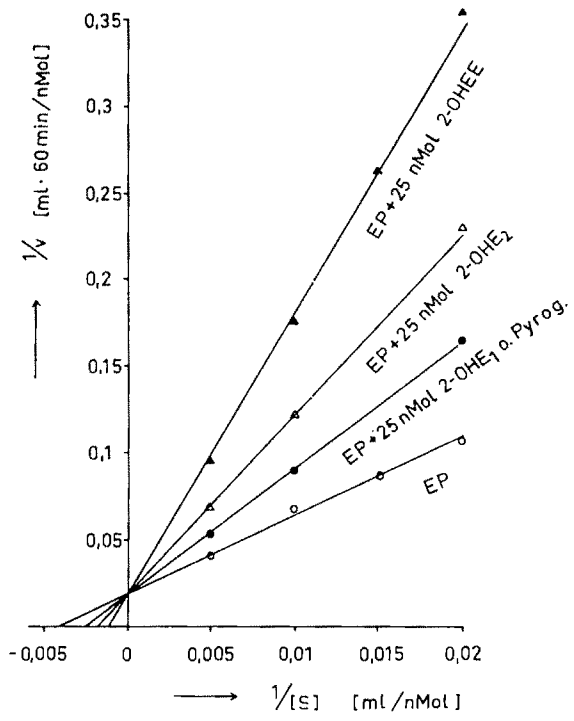


Fig. 4. Methylation of epinephrine by rat liver cytosol without and with addition of different inhibitors.

rat liver homogenates metabolize monophenolic estrogens to water-soluble metabolites that are not glucuronides or sulfates.[25] One possibility is that the further hydroxylation of catecholestrogens leads to tetrahydroxylated, highly water-soluble compounds; another possibility is the irreversible binding to amino acids and proteins, including the formation of thioethers.

With respect to thioether formation, Jellinck et al.[26] and Kuss[27] observed that rat liver microsomes in the presence of the soluble fraction[26] or a rat liver homogenate[27] transformed E_2 to metabolites that had the characteristics of both a peptide and a phenolic steroid. The isolated products were mainly glutathione conjugates coupled with the steroid by a carbon-sulfur linkage, predominantly at C-atom 4 (ratio of the 4- to the 1-S isomer approximately 3 : 1). Because the same products were also formed when microsomes or homogenate were completely omitted, a spontaneous nonenzymatic process (the addition of thiol by redox reactions) was assumed (Fig. 5), and it was stated that the semiquinone radical of catecholestrogens seems to be the molecular species immediately preceding the thioether.[5] Moreover, because the in vivo formation of thioethers was never shown,[5] it was concluded that "activated estrogens," capable of reacting spontaneously with glutathione, are formed only in very artificial systems.[5]

Carcinogenesis and cell damage hypothetically induced by estrogens, especially EE_2 and diethylstilbestrol,[28] have been proposed to be mediated by activated catecholestrogen metabolites[29-33]; activated catecholestrogens are known to react covalently with protein and nucleic acids. However, the mechanism of interaction remains obscure: sulfydryl groups as acceptors have been excluded, whereas catecholestrogen-protein thioethers have not been found.[5] Thus amino groups of nucleic acid bases or proteins may react with catecholestrogen quinones. Glutathione, ascorbic acid, and lipids are thought to compete with macromolecular binding, thus possibly preventing some of the deleterious effects of catecholestrogens.[5]

I now come to binding, with functional preservation of the catechol structure, that is, binding to receptor and plasma proteins.

Aside from production and metabolism, the availability of steroids is determined by their binding to plasma proteins. In humans steroid hormones circulate in complex with three major plasma proteins: sex hormone–binding globulin, cortisol-binding globulin, and albumin.[34] Although the relative binding to the three proteins varies widely from steroid to steroid, sex hormone–binding globulin is the most important binding protein. The relative binding affinities given in Table V show a striking affinity of 2-OCE_2 that exceeds that of E_2 by a factor of 4, whereas the relative binding affinity of catecholestrogens amounts to about 1% of that value.

The finding that 2-methylated catecholestrogens bind to sex hormone–binding globulin with high affinity in vitro and that sex hormone–binding globulin is the major transport protein for sex steroids in vivo

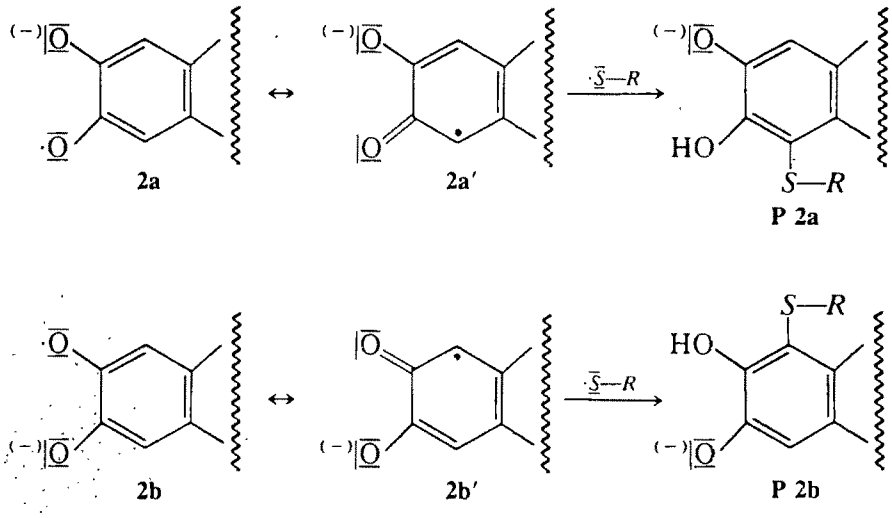


Fig. 5. Proposed mechanism of thioether formation. (From Kuss E. Estrogens and glutathione. In: Dolphin D, Voulson R, Avramovic O, eds. Glutathione. New York: John Wiley & Sons, 1989:512-49. Copyright© 1989 by John Wiley & Sons, Inc. Reprinted by permissions of John Wiley & Sons, Inc.)

Table V. Relative binding affinities of 2- and 4-substituted estrogens to sex-hormone–binding globulin (testosterone = 1000)

2-OCE_2	2000
Testosterone	1000
2-OCE_1	750
Estradiol	500
2-OHE_2	7

raises the possibility that compounds that have negligible sex hormone activity could exert physiologically important effects by altering the transport of other endogenous sex hormones. With a computer model, the tremendous impact of increasing 2-OCE_1 concentrations on free E_2 and testosterone concentrations was calculated.[34]

Assuming that catecholestrogens bind to the same receptor as E_2, Martucci and Fishman[35] examined the relative binding affinities of a series of 2- and 4-hydroxylated estrogens compared with E_2. In the crude uterine receptor preparation used, that is, 120,000 *g* supernatant of rat uterus homogenate, the percentages shown in Table VI were obtained.

To evaluate the biologic significance of these binding affinities, some further investigations must be mentioned.[3] Comparing the biologic potencies after short-term injection with these relative binding affinities, the following relationships have been found: 2-OHE_1 has 20% of the relative binding of E_1 but only 0.1% of its estrogenic potency (ratio 1:200), and 4-OHE_2 has 50% of the relative binding of E_2 but only 10% of its estrogenic potency (ratio 1:5). The reasons for these discrepancies are as follows: (1) 2-OHE_1 is more intensely metabolized than is 4-OHE_2 and especially E_2, thus preventing comparable amounts of the steroid ever to reach the receptor in vivo.[3] (2) Assuming that catechol estrogens might act analogous to E_3, in vivo dissociation rates were examined. As anticipated, 2-hydroxylation resulted in an increase, but 4-hydroxylation resulted in a decrease in dissociation rates compared with the parent estrogen. Consequently, an interesting experiment was done.[36] In an attempt to minimize the effects of differences in the rates of metabolism, two successive injections of estrogens in doses adjusted to give similar nuclear estrogen receptor concentrations were applied to living rats and the biologic effect (concentrations of the cytoplasmic progestin receptor in the rat pituitary gland) was monitored (Table VII). Strikingly, 2-OHE_2 was about one half less, but 4-OHE_2 was about one half more potent than the parent E_2.

In conclusion, on continous administration, differences in metabolism, receptor binding, receptor kinetics, and biologic responses lead to a much smaller biologic (estrogenic) effect of 2-hydroxyestrogens but to about an equal effect of E_2 and 4-OHE_2.

Interactions of catecholestrogens (especially EE) with catecholamines, proteins, and deoxyribonucleic acid

In general, it may be stated that the production and endogenous metabolism of estrogens are strongly interconnected with the metabolism and biologic effects of other chemically related endogenous and exogenous substances.

From the literature it is known that the biologic effects of catecholamines are governed not only by enzymatic formation (key enzyme: tyrosinase), enzymatic inactivation (COMT/monoamine oxidase), and ultimate receptor binding, but also by the reuptake of

Table VI. Relative binding affinities of estrogens to the cytosolic receptor (percent)

E_2	100
4-OHE_2	43
2-OHE_2	23
E_1	11
4-OHE_1	10
2-OHE_1	2
2-OCE_2	0.05
2-OCE_1	0.01

Table VII. Biologic effect of estrogens in rat pituitary

Compound	*Cytoplasmic progestin receptor (fm/mg protein)*
Vehicle	6.4
2-OHE_2	12.7
E_2	25.9
4-OHE_2	32.1

Table VIII. Interaction of estrogens with catecholamine physiology

	Inhibition
CA reuptake	17a-EE > E_2 > 2-OHE > 4-OHE
Tyrosinase	NE = 2-OHE_2; E_2 inactive
COMT	17a-EE > 2-OHE_2 > 2-OHE_1
CA receptors	E_2 > 2-OHE_2

CA, Catecholamine; *NE*, norepinephrine.

catecholamines into the cell. EE could possibly interact with all four events.

Without discussing the details of the investigations reported so far, the following may be summarized[37] (Table VIII): the interference of E_2 and 2-OHE_2 with the catecholamine receptor is at least questionable.

Catecholestrogens as catecholamines are approximately equal potent inhibitors of tyrosinase activity. However, because high concentrations of catecholestrogens are necessary to cause measurable inhibition, the physiologic role of this interaction remains uncertain.

Although in vitro catecholestrogens and especially 2-OHEE are potent inhibitors of the COMT-mediated inactivation of catecholamines, this inhibiton with its proved prolongation of catecholamine action does not seem essential for the regulation of blood pressure in vivo. As suggested by Ghraf and Hiemke,[37] the reuptake inhibition brought about by E_2 and 2-OHE_2 may be the dominant mechanism.

17α-EE is a potent inhibitor of catecholamine reuptake. The introduction of a 2-hydroxy- and especially the 4-hydroxy group, however, reduces the inhibitory potency.

In conclusion, it may be stated that EE, together with its ortho-hydroxylated metabolites (2-OHEE and 4-OHEE), increases the biologic activity of catecholamines more than the primary estrogens, an effect that may be enhanced even during high catecholamine secretion states, such as under the influence of stress caused by physical activity.[38]

Another possibly adverse effect of the highly effective synthetic estrogen compared with primary estrogens may stem from its more rapid metabolism to catechol derivatives, with the potential of irreversible binding to cellular macromolecules such as proteins and deoxyribonucleic acid. It is interesting to note that the oncogenic potential of an estrogen seems to be related to its relative rate of conversion to a catecholestrogen and not to its estrogenic potency per se.[39] Although controversial in the literature,[28] high concentrations of estrogens, but especially of EE, present over a long time interval may eventually facilitate the development of cancer, especially uterine and breast cancers.

REFERENCES

1. Ball P, Haupt M, Knuppen R. Comparative studies on the metabolism of oestradiol in the brain, the pituitary and the liver of the rat. Acta Endocrinol 1978;87:1-11.
2. Ball P, Farthmann E, Knuppen R. Comparative studies on the metabolism of oestradiol-17β and 2-hydroxyoestradiol-17β in man in vitro and in vivo. J Steroid Biochem 1976;7:139-43.
3. Ball P, Knuppen R. Catecholoestrogens. Acta Endocrinol Suppl 1980;232:1-127.
4. Emons G, Ball P, Knuppen R. Radioimmunoassays of catecholestrogens. In: Merriam GR, Lipsett MB, eds. Catecholestrogens. New York: Raven Press, 1983:71-81.
5. Kuss E. Estrogens and glutathione. In: Dolphin D, Voulson R, Avramovic O, eds. Glutathione. New York: John Wiley & Sons, 1989:512-49.
6. Levin M, Weisz J, Bui QD, Santen RJ. Peroxidatic catecholestrogen production by human breast cancer tissue in vitro. J Steroid Biochem 1987;28:513-20.
7. Dannan GA, Porubek DJ, Nelson SD, Waxman DJ, Guengerich FP. 17β-estradiol, 2- and 4-hydroxylation catalyzed by rat hepatic cytochrome P-450: roles of individual forms, inductive effects, developmental patterns, and alterations by gonadectomy and hormone replacement. Endocrinology 1986;118:1952-60.
8. Shiverick KT, Notelovitz M. Regulation of cytochrome P-450–dependent catechol estrogen formation in rat liver microsomes. Biochem Pharmacol 1983;32:2399-403.
9. Nocke-Finck L, Breuer H, Reimers D. Wirkung von Rifampicin auf den Menstruationzyklus und die Östrogenausscheidung bei Einnahme oraler Kontrazeptiva. Dtsch Med Wochenschr 1973;98:1521-3.
10. Bolt HM, Kappus H, Bolt M. Effect of rifampicin treatment on the metabolism of oestradiol and 17-α-ethynyloestradiol by human liver microsomes. Eur J Clin Pharmacol 1975;8:301-7.
11. Brueggemeier RW, Kimball JG. Kinetics of inhibition of estrogen 2-hydoxylase by various haloestrogens. Steroids 1983;42:93-103.
12. Quail JA, Jellinck PH. Modulation of catechol estrogen synthesis by rat liver microsomes: effects of treatment with growth hormone or testosterone. Endocrinology 1987; 121:987-92.
13. Purdy RH, Moore PH, Williams MC, Goldzieher JW, Paul SM. Relative rates of 2- and 4-hydroxyestrogen synthesis are dependent on both substrate and tissue. FEBS Lett 1982;138:40-4.

14. Axelrod J, Tomchick R. Enzymatic O-methylation of epinephrine and other catechols. J Biol Chem 1958;233: 702-5.
15. Knuppen R, Breuer H, Pangels G. Stoffwechsel von 2-Hydroxyöstradiol-(17β) und 2-Methoxy-östradiol-(17β) in Geweben des Menschen und der Ratte. Hoppe-Seyler's Z Physiol Chem 1961;324:108-17.
16. Ball P, Knuppen R, Breuer H. Purification and properties of a catechol-O-methyltransferase of human liver. Eur J Biochem 1971;21:517-25.
17. Ball P, Haupt M, Knuppen R. Biogenesis and metabolism of catechol estrogens in vitro. In: Merriam GR, Lipsett MB, eds. Catecholestrogens. New York: Raven Press, 1983:91-103.
18. Ball P, Knuppen R, Haupt M, Breuer H. Kinetic properties of a soluble catechol O-methyltransferase of human liver. Eur J Biochem 1972;26:560-9.
19. Ball P, Knuppen R, Haupt M, Breuer H. Interactions between estrogens and catechol amines III. Studies on the methylation of catechol estrogens, catechol amines and other catechols by the catechol O-methyltransferase of human liver. J Clin Endocrinol Metab 1972;34:736-46.
20. Ball P, Gelbke HP, Haupt O, Knuppen R. Metabolism of 17-α-ethynyl-4^{14}C oestradiol and 4-^{14}C mestranol in rat liver slices and interaction between 17-α-ethynyl-2-hydroxyoestradiol and adrenaline. Hoppe-Seyler's Z Physiol Chem 1973;354:1567-75.
21. Ball P, Knuppen R, Wennrich W, Breuer H. Interactions between oestrogens and catecholamines: influence of oestrogens on the effect of catecholamines on blood pressure in rats. Acta Endocrinol Suppl 1972;159:85.
22. Bates GW, Edman CD, Porter JC, MacDonald PC. Metabolism of catechol estrogen by human erythrocytes. J Clin Endocrinol Metabol 1977;45:1120-3.
23. Lipsett MB, Merriam GR, Kono S, Brandon DD, Pfeiffer DG, Loriaux DL. Metabolic clearance of catechol estrogens. In: Merriam GR, Lipsett MB, eds. Catecholestrogens. New York: Raven Press, 1983:105-14.
24. Ball P, Hoppen H-O, Knuppen R. Metabolism of oestradiol-17β and 2-hydroxyoestradiol-17β in rat liver slices. Hoppe-Seyler's Z Physiol Chem 1974;355:1451-62.
25. Riegel IL, Mueller GC. Formation of a protein-bound metabolite of estradiol-16-C^{14} by rat liver homogenates. J Biol Chem 1954;210:249-57.
26. Jellinck PH, Lewis J, Boston F. Further evidence for the formation of an estrogenpeptide conjugate by rat liver in vitro. Steroids 1967;10:329-46.
27. Kuss E. Wasserlösliche Metabolite des 17β-Östradiols. Hoppe-Seyler's Z Physiol Chem 1967;348:1707-1708.
28. Metzler M. Metabolism of stilbene estrogens and steroidal estrogens in relation to carcinogenicity. Arch Toxicol 1984;55:104-9.
29. Rajan R, Daly MJ, Reddy VVR. Estrogen effects on NADH oxidase and superoxide dismutase in prepubertal female rats. Steroids 1982;40:651-60.
30. Vandewalle B, Peyrat J-P, Bonneterre J, Lefebvre J. Catecholestrogen binding sites in breast cancer. J Steroid Biochem 1985;23:603-10.
31. Liehr JG, Ulubelen AA, Strobel HW. Cytochrome P-450–mediated redox cycling of estrogens. J Biol Chem 1986; 261:16865-70.
32. Liehr JG, Fang WF, Sirbasku DA, Ari-Ulubelen A. Carcinogenicity of catechol estrogens in Syrian hamsters. J Steroid Biochem 1986;24:353-6.
33. Aswegen CH, Purdy RH, Wittliff JL. Binding of 2-Hydroxyestradiol and 4-Hydroxyestradiol to estrogen receptors from human breast cancers. J Steroid Biochem 1989;32:485-92.
34. Dunn JF. Transport of estrogens in human plasma. In: Merriam GR, Lipsett MB, eds. Catechol estrogens. New York: Raven Press, 1983:167-76.
35. Martucci Ch, Fishman J. Uterine estrogen receptor binding of catecholestrogens and of estetrol (1,3,5(10)-estratriene-3,15a,16a,17β-tetrol). Steroids 1976;27:325-33.
36. MacLusky NJ, Barnea ER, Clark CR, Naftolin F. Catechol estrogens and estrogen receptors. In: Merriam GR, Lipsett MB, eds. Catechol estrogens. New York: Raven Press, 1983:151-65.
37. Ghraf R, Hiemke Ch. Interaction of catechol estrogens with catecholamine synthesis and metabolism. In: Merriam GR, Lipsett MB, eds. Catechol estrogens. New York: Raven Press, 1983:177-87.
38. Keizer HA, Kuipers H, Verstappen FTJ, Janssen E. Limitations of concentration measurements for evaluation of endocrine status of exercising women. Can J Appl Spt Sci 1982;7:79-84.
39. Purdy RH, Goldzieher JW, Le Quesne PW, Abdel-Baky S, Durocher CK, Moore PH Jr. Active intermediates and carcinogenesis. In: Merriam GR, Lipsett MB, eds. Catechol estrogens. New York: Raven Press, 1983:123-40.

SESSION IV. HORMONAL EFFECTIVENESS OF ORAL CONTRACEPTIVE STEROIDS

Chairman: M. Breckwoldt

Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor

Michael Juchem* and Kunhard Pollow, MD, PhD
Mainz, West Germany

Some progestogens widely used in oral contraceptives are characterized at the level of high-affinity receptor binding as well as binding to sex hormone–binding globulin and corticosteroid-binding globulin. With regard to binding to sex hormone–binding globulin, gestodene, levonorgestrel, and to a lesser extent 3-ketodesogestrel (which is only formed from the prodrug desogestrel in the body), show a behavior that is manifested in the relatively high affinity to sex hormone–binding globulin, whereas desogestrel and norgestimate do not display any measurable affinity for this specific steroid-binding serum protein. Furthermore, levonorgestrel and gestodene dissociate very much more slowly from the binding sites of sex hormone–binding globulin than 3-ketodesogestrel. A natural affinity of all these synthetic progestogens tested for corticosteroid-binding globulin could not be established. Gestodene, levonorgestrel, and 3-ketodesogestrel bind to the progesterone, glucocorticoid, and androgen receptor with high affinity, apart from slight differences, whereas estrogen receptor affinity could not be demonstrated in any of the progestogens investigated. In relation to aldosterone, the relative binding affinity values of gestodene, levonorgestrel, and the natural progestogen progesterone are relatively high, whereas 3-ketodesogestrel does not display any measurable affinity for this receptor species. (Am J Obstet Gynecol 1990;163: 2171-83.)

Key words: Sex hormone–binding globulin, competition studies, steroid receptors, gestodene, desogestrel, levonorgestrel, norgestimate

A generally accepted classical principle of therapy with pharmacologically active substances is that the greatest possible effectiveness is to be reached at the lowest dosage. The idea behind this approach is based on the assumption that the lower the therapeutic dose, the less the burden on the body as a whole, especially in terms of possible adverse drug effects.

With regard to oral contraceptives, efforts have been made over many years to reduce the overall amount of estrogenically and progestogenically active steroid components applied over the menstrual cycle and to find synthetic steroids that offer not only efficacy but also a large measure of tolerance.

The proportion of estrogen in the oral contraceptives of the most recent generation has been reduced; for example, it is down to 30 μg per pill in the most widespread form of ethinyl estradiol, above all to lower markedly the incidence of adverse drug effects caused by estrogens. Regarding the gestagen constituent in oral contraceptives, however, there is no uniformity with regard to the steroid used or to the dosage. The synthetic progestogens most frequently used are levonorgestrel, desogestrel, gestodene, cyproterone acetate, and norgestimate. Thus levonorgestrel, the classic orally active synthetic progestogen, presents the advantage of balancing overshoot and adverse effects of the estrogen component besides providing a markedly reduced dosage.[1] The antiandrogenically active cyproterone acetate had the advantage of processing a highly antiandrogenic activity component, in addition to its potent gestagenic effects, which allowed an additional effective treatment in androgenization manifestations of the skin in potential users of oral contraceptives.[2-5] Desogestrel,[6-9] gestodene,[10-13] and norgestimate,[14] further agents from the 19-nortestosterone series, which are now available for clinical application in oral contraceptives, appeared to set new standards with regard to activities and hormonal profiles.

The dosage of progestogens used in oral contraceptives shows a large range of variation. However, a dose reduction of a certain progestogen is limited by its hormonal effectiveness, because the component used in oral contraceptives has to suppress ovulation reliably.

From the Department of Experimental Endocrinology, Johannes Gutenberg University.
Reprint requests: Kunhard Pollow, MD, PhD, Department of Experimental Endocrinology, Johannes Gutenberg University, Langenbeckstrasse 1, 6500 Mainz 1, Federal Republic of Germany.
**This work is part of the PhD thesis of M. Juchem.*
6/0/24390

Table I. Relative binding affinities (%) of various progestogens in comparison with cortisol and DHT for binding to human CBG and SHBG

	CGB	*SHBG*
Cortisol	100	<0.1
DHT	<0.1	100
Progesterone	53	<0.1
Medroxyprogesterone acetate	<0.1	<0.1
Cyproterone acetate	<0.1	<0.1
Org2058	<0.1	<0.1
R5020	<0.1	<0.1
Levonorgestrel	<0.1	50
Gestodene	<0.1	40
3-Ketodesogestrel	<0.1	5
Desogestrel	<0.1	<0.1
Norgestimate	<0.1	<0.1

Therefore, a progestogen used in a lower dose must be more potent, not only with respect to central effects, but also in regard to peripheral effects.

The efficacy of the progestogen used is a feature consisting of a kaleidoscope of the most different qualities. According to the conventional view, a basic prerequisite for gestagenic activity is an adequate affinity for the progesterone receptor and active induction of a conformational change of the receptor molecule during binding as well as the duration of binding to the chromatin. Whereas the affinity of a progestogen to the receptor is a parameter determined by the structure of the steroid, the number of the receptors occupied by the steroids depends on the steroid concentration in the target cell. The intracellular steroid level is in turn a function of the "free" steroid, which is only able to enter the cell, but which is also dependent on intracellular metabolism and storage. The blood level of "free" steroid, that is, nonprotein-bound steroid, is mainly influenced by the total steroid level and by the affinity to specific steroid-binding proteins such as sex hormone–binding globulin (SHBG) or corticosteroid-binding globulin (CBG).

The blood levels also depend on absorption, metabolism in the gastrointestinal tract and in the liver, distribution and storage in the tissues, the enterohepatic circulation, and the rate of elimination and excretion-dependent function, that is, the pharmacokinetics. The dosage of progestogens in oral contraceptives is thus only one link in the long chain that ultimately determines the efficacy of a steroid hormone.

The aim of the present study is to make a broad characterization of progestogens such as levonorgestrel, desogestrel, gestodene, and norgestimate,[1-14] which are widely used in oral contraceptives, at the level of receptors and as high-affinity steroid-binding serum proteins.

Material and methods

Materials. Tritiated aldosterone (specific activity 2.96 terabecquerel [TBq]/mmol), tritiated R5020 (specific activity 3.22 TBq/mmol), tritiated DHT (specific activity 5.29 TBq/mmol), tritiated estradiol (specific activity 3.44 TBq/mmol), tritiated R1881 (methyltrienolone) (specific activity 3.22 TBq/mmol), and tritiated unlabeled R5020 and R1881 were purchased from New England Nuclear, Dreieich, West Germany. Tritiated dexamethasone (specific activity 3.03 TBq/mmol), tritiated Org2058 (specific activity 2.2 TBq/mmol), and tritiated cortisol (specific activity 3.6 TBq/mmol) were obtained from Amersham Buchler, Braunschweig, West Germany. Tritiated gestogene (specific activity 2.0 TBq/mmol), tritiated 3-ketodesogestrel (specific activity 1.6 TBq/mmol), tritiated levonorgestrel (specific activity 1.8 TBq/mmol), and unlabeled gestodene, desogestrel, 3-ketodesogestrel, norgestimate and levonorgestrel were a gift from Schering AG, Berlin-Bergkamen, West Germany. Other steroids used were ordered from Serva, Heidelberg, West Germany. All other chemicals were of pharmaceutical grade. PENG-buffer consisted of 10 mmol/L KH_2PO_4, 10 mmol/L K_2HPO_4, 1.5 mmol/L EDTA, 3 mmol/L NaN_3, 5 mmol/L monothioglycerol, and 10% glycerol, pH 7.5. DCC (dextran-coated charcoal) consisted of 0.5% Norit A and 0.05% dextran T400 in PENG-buffer.

Tissues and serum samples. Kidneys of adrenalectomized female Wistar rats were used as a source for mineralocorticoid receptor. Estrogen and progesterone receptors were assayed in cytosols of human uterine tissues. Glucocorticoid receptors were determined in cytosols of adrenalectomized female Wistar rat livers. The binding of steroids toward androgen receptors was determined in the cytosol of prostates of castrated rats.

For binding analysis to the serum steroid-binding proteins (CBG and SHBG), human pregnancy serum that was stripped of endogenous steroids with dextran-coated charcoal was used. All tissues and serum samples were quick-frozen in liquid nitrogen and stored at −80° C until use.

Cytosol preparation. The frozen tissue samples were minced, and after the addition of cold PENG-buffer, were homogenized (Ultra Turrax; Janke und Kunkel, Melsungen, West Germany).

The homogenate was centrifuged at 105,000 *g* at 4° C for 30 minutes in a fixed-angle rotor (60 Ti; Beckman Instruments, Munich, West Germany). The clear supernatant was used. Protein concentrations of the cytosols were determined according to the method of Lowry et al.[15]

Relative binding affinities

MINERALOCORTICOID RECEPTORS. Aliquots (0.1 ml) of rat kidney cytosols were incubated with tritiated al-

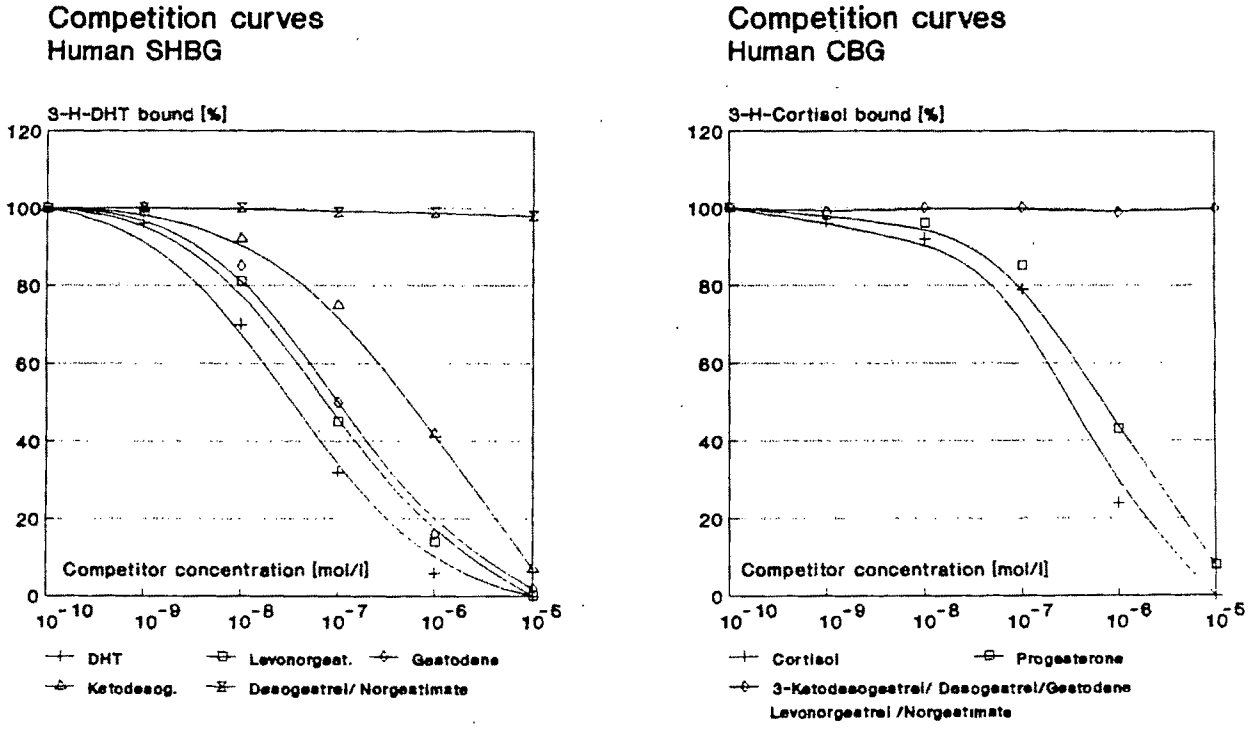


Fig. 1. Competition for labeled DHT and cortisol binding in diluted serum of pregnant women.

dosterone (50 μl, final concentration 8 nmol/L), together with increasing concentrations (50 μl, final concentration 10^{-10} to 10^{-5} mol/L) of various competitor steroids for 4 hours at 4° C in the presence of a 200-fold molar excess of nonlabeled RU 28362. Unbound steroids were adsorbed by incubating with 0.5 ml of a dextran-coated charcoal suspension for 10 minutes at 4° C. After centrifugation (10 minutes, 1500 *g*, 4° C), 0.5 ml of the supernatant was withdrawn and counted for radioactivity. The relative binding affinities were evaluated according to the method of Korenman.[16]

GLUCOCORTICOID RECEPTORS. Cytosols of livers of adrenalectomized rats were used, and tritiated dexamethasone (final concentration 8 nmol/L) served as ligand. The incubation period was 4 hours at 4° C.

PROGESTERONE RECEPTORS. For cytosol preparation, human uterine tissues were used; tritiated R5020 (final concentration 8 nmol/L) served as ligand. The incubation period was overnight at 4° C.

ESTROGEN RECEPTORS. Cytosol was prepared from human uterine tissues; the ligand was tritiated estradiol (final concentration 8 nmol/L). The incubation period was overnight at 4° C.

ANDROGEN RECEPTORS. Progesterone receptor-free cytosols of prostates of castrated rats were used; tritiated R1881 (final concentration 8 nmol/L) served as ligand in the presence of a 200-fold molar excess of unlabeled triamcinolone acetonide. The incubation period was 4 hours at 4° C.

SERUM BINDING PROTEINS. Relative binding affinities toward SHBG and CBG were determined by using stripped human pregnancy serum in a dilution with PENG-buffer in a ratio of 1:50 and tritiated cortisol or tritiated DHT as ligands, both in a final concentration of 8 nmol/L. The incubation period was overnight at 4° C.

Rate of association of tritium-labeled progestins with serum proteins. Aliquots (0.1 ml) of diluted serum from pregnant women or purified SHBG were incubated in triplicate with different tritium-labeled progestins (final concentration 8 nmol/L), either alone (total binding) or together with a 200-fold molar excess of unlabeled identical progestin at 4° C for different time intervals as indicated in the Figures. At the time intervals indicated, 0.5 ml dextran-coated charcoal suspension was added and incubated for 10 minutes. After centrifugation (10 minutes, 1500 *g* 4° C), 0.5 ml of the supernatant was withdrawn and counted for radioactivity.

Rate of dissociation of different tritium-labeled progestins from serum proteins. Aliquots (0.1 ml) of diluted serum from pregnant women, or purified SHBG and CBG, were incubated in triplicate with tritium-labeled progestins (final concentration 8 nmol/L) plus or minus a 200-fold molar excess of unlabeled identical progestin overnight at 4° C. To all tubes 0.5 ml of dextran-coated charcoal was added and incubated for different time intervals, as indicated in the Figures, under gentle shaking at 4° C. After the

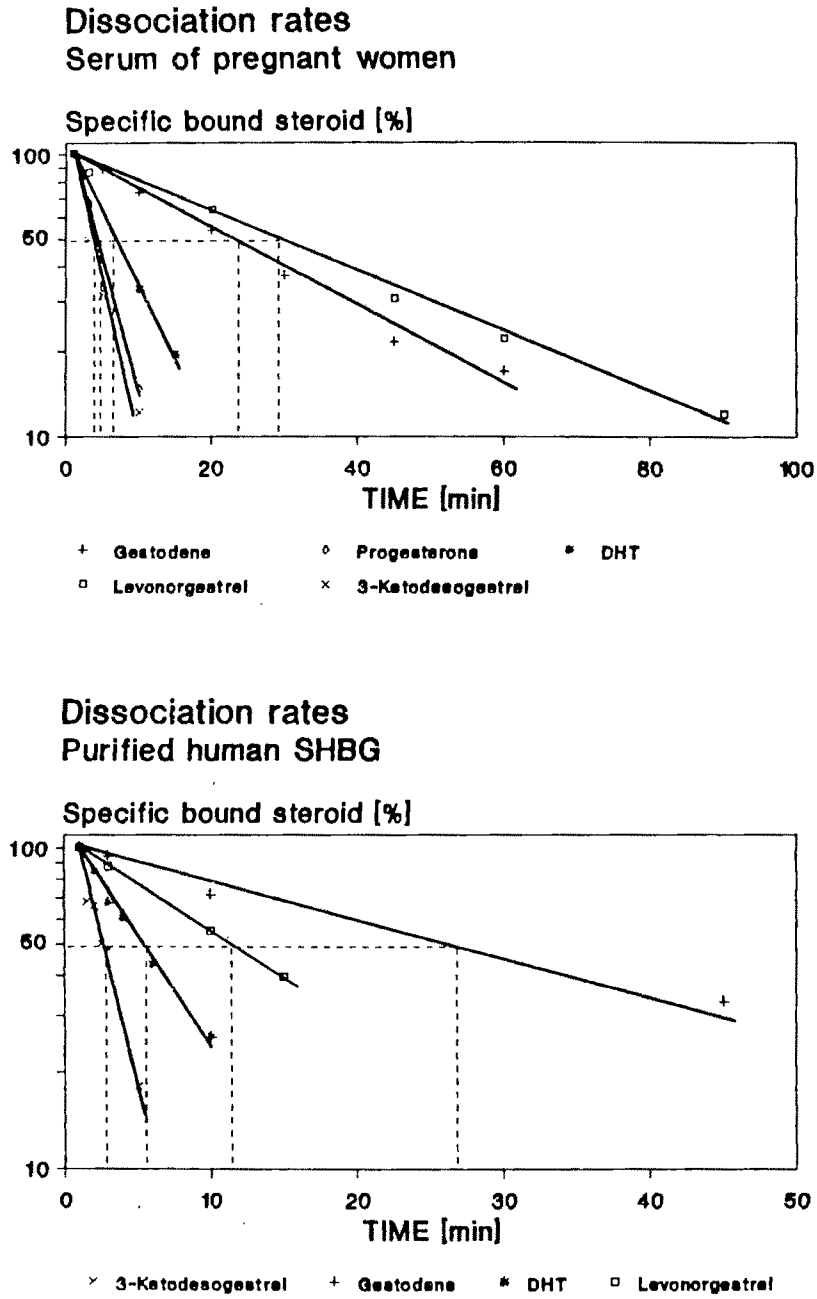


Fig. 2. Determination of the half-life of various tritium-labeled steroid-binding complexes of diluted serum of pregnant women and purified human SHBG.

time intervals indicated, the tubes were centrifuged (10 minutes, 1500 *g*, 4° C), and 0.5 ml of the supernatant was counted for radioactivity.

Titration analyses. The general set-up was as follows: 0.1 ml aliquots of aqueous tritiated steroid solutions (final concentrations 0.5, 1.0, 2.0, 4.0, and 8.0 nmol/L) were incubated either alone (total binding, duplicates) or together with a 200-fold molar excess of unlabeled competitor (nonspecific binding), together with 0.1 ml of diluted serum of pregnant women for 4 hours at 4° C. Dextran-coated charcoal (0.5 ml) was added, incubated for 10 minutes or only 5 minutes at 4° C and centrifuged for 10 minutes at 1500 *g* and 4° C. Aliquots (0.5 ml) of the supernatants were withdrawn and counted for radioactivity. The binding data were calculated according to the method of Scatchard.[17]

Sucrose density gradient analysis. Aliquots of 0.5 ml of human uterine cytosol were incubated with 16 nmol/L of tritium-labeled steroids with or without a 200-fold molar excess of unlabeled steroids for 16

hours at 4° C. After that, unbound steroids were then removed by DCC treatment and 0.2 ml aliquots were layered on top of 10% to 35% linear sucrose gradients. The gradients were centrifuged for 2.5 hours at 4° C at 404,000 *g* in a vertical rotor (Beckman VTi 65). The gradients were collected from the bottom in 40 fractions and were standardized with the following proteins: fluorescein isothiocyanate–labeled bovine serum albumin (molecular weight 67,000) and fluorescein isothiocyanate–labeled immunoglobulin G (molecular weight 150,000) (Behring Werke, Marburg, West Germany).

Purification of SHBG and CBG. Human SHBG or CBG was purified to homogeneity by application of three different chromatographic methods as previously described in detail.[18, 19]

Results

Serum proteins

Competition experiments. The determination of the relative binding affinities (RBA values) allows an evaluation of the specific interaction between a ligand and the binding site of steroid-binding serum proteins such as CBG and SHBG or of intracellular receptor proteins in relation to a radioactively labeled standard ligand typical of the respective binding protein. Table I and Fig. 1 summarize the results of the competition behavior of progesterone as compared with different established synthetic progestogens tested on specific serum proteins. In contrast to progesterone, which binds to CBG with an RBA value of 53% when compared with cortisol (100%), a natural affinity of all synthetic progestogens for CBG could not be established. With regard to binding to SHBG, gestodene, levonorgestrel—and to a lesser extent 3-ketodesogestrel—show a behavior that is typical of 19-nortestosterone derivatives and that is manifested in the relatively high affinity to SHBG when compared with DHT (100%). The RBA value for levonorgestrel is 50%, for gestodene 40%, and for 3-ketodesogestrel, 5%, whereas progesterone does not bind to SHBG. The synthetic progestogens from the C-21 series, medroxyprogesterone acetate and cyproterone acetate, along with the highly potent synthetic ligands for progesterone receptor analysis, Org2058 and R5020, are characterized by a lack of binding to both steroid-binding serum proteins. The same is also true for norgestimate.

Association and dissociation rates. Kinetic parameters such as association and dissociation rates of steroid hormone serum protein complexes provide important information as to the stability of such complexes.

The dissociation of different steroid hormones from the binding areas of serum proteins was measured by treatment of diluted serum of pregnant women or purified human SHBG and CBG with dextran-coated charcoal that had been incubated with the corresponding tritium labeled steroid under conditions of saturation. Fig. 2 and Table II document that levonorgestrel and gestodene dissociate much more slowly, both from the binding sites of pregnancy serum and from the binding sites of purified SHBG, than 3-ketodesogestrel and DHT (which was used as a reference steroid for the specific detection of SHBG). This is a manifestation of relatively stable steroid-protein complexes.

Table II. Dissociation rates of different steroid hormone serum protein complexes

Steroid	*Diluted serum of pregnant women*	*Purified SHBG*	*Purified CBG*
Progesterone	4.4	n.m.	4.8
DHT	6.6	5.1	n.m.
Levonorgestrel	29.3	11.8	n.m.
Gestodene	23.8	26.4	n.m.
3-Ketodesogestrel	3.8	2.6	n.m.

Values are half-life minutes.
n.m., Not measured.

Table III. Association rates (80% values) of different steroid hormones with specific binding components of diluted serum of pregnant women and purified SHBG

Steroid	*Diluted serum of pregnant women*	*Purified SHBG*
Progesterone	12	n.m.
DHT	12	90
Levonorgestrel	12	18
Gestodene	12	18
3-Ketodesogestrel	>>240	>>240

Values are association rates in minutes.
n.m., Not measured.

The kinetics of the association of different tritium-labeled steroid hormones with the specific binding sites of serum proteins over a period of 4 hours are shown in Table III and Fig. 3. After saturation of the binding sites from diluted serum of pregnant women or purified SHBG with tritium-labeled gestodene, levonorgestrel, and DHT within 12 minutes (80%) values, the specific binding remains stable over a period of at least 4 hours at 4° C. The association kinetics of 3-ketodesogestrel established in pregnancy serum or purified SHBG demonstrate that there was no saturation of the binding sites even after 4 hours of incubation under saturation conditions.

Scatchard plot analysis. The titrations of the specific binding sites with DHT, gestodene, levonorgestrel, and 3-ketodesogestrel in diluted human serum of pregnant

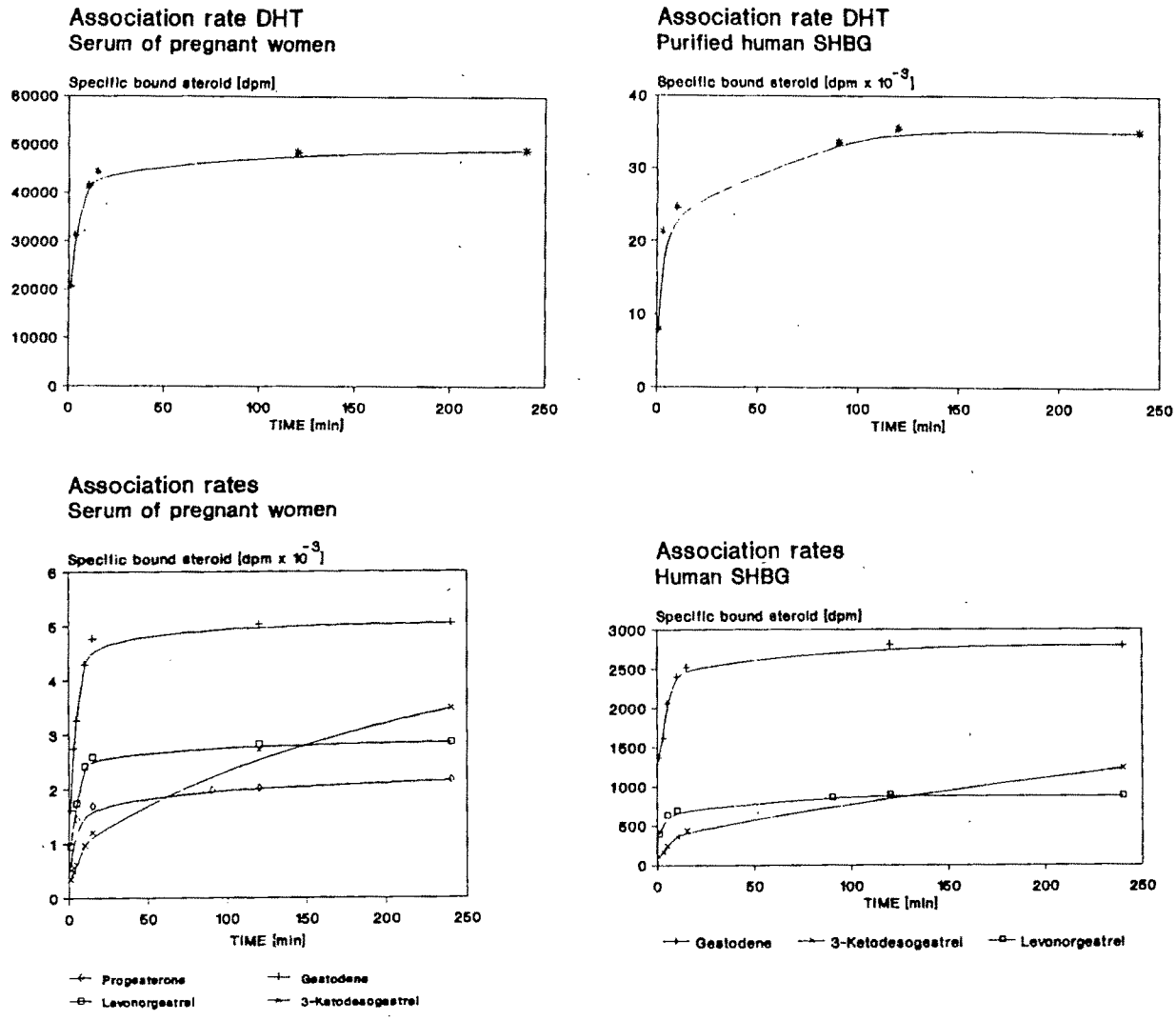


Fig. 3. Time course of association of various tritium-labeled steroids with diluted serum of pregnant women and purified human SHBG.

Table IV. Determination of K_d values according to Scatchard of different steroids in diluted serum of pregnant women

Steroid	*K_d values*
DHT	1.9×10^{-9} mol/L after 10-minute DCC treatment
Levonorgestrel	1.3×10^{-8} mol/L after 10-minute DCC treatment
Gestodene	1.0×10^{-8} mol/L after 10-minute DCC treatment
3-Ketodesogestrel	n.m. after 10-minute DCC treatment
3-Ketodesogestrel	1.9 ± 10^{-8} mol/L after 5-minute DCC treatment

n.m., Not measured.

women show that after transformation of the binding data according to Scatchard there is a typically linear correlation between bound/free and tritium-labeled ligand concentrations. The dissociation constants (K_d values), determined arithmetically from the Scatchard plot analysis, are $K_d = 1.9 \times 10^{-9}$ mol/L for DHT, whereas the K_d values for gestodene and levonorgestrel are one order of magnitude lower under conditions of 10 minutes of DCC treatment (Table IV). In contrast to this, a titration according to Scatchard is not possible owing to the extreme lability of the steroid-protein complex when 3-ketodesogestrel is used as a ligand under the condition of 10 minutes of DCC treatment for separation of free and protein-bound steroid. Only the reduction of the incubation time with DCC to 5 minutes allows an arithmetic determination of the binding data according to Scatchard ($K_d = 1.9 \times 10^{-8}$ mol/L) in this case.

Receptor studies

Competition experiments. Table V summarizes the results of competition experiments on the level of progesterone receptor from human uterine tissues with various reference steroids. R5020 is the reference

Table V. Relative binding affinities (%) of different progestogens for binding to human uterine progesterone receptor

	R5020	*Ketodesogestrel*	*Gestodene*	*Levonorgestrel*
Progesterone	40	15	2	18
Gestodene	85	110	100	150
Levonorgestrel	120	90	75	100
R5020	100	20	20	22
Org2058	350	n.d.	35	n.d.
Desogestrel	1	0.3	<0.1	0.3
3-Ketodesogestrel	130	100	55	110
Norgestimate	<0.1	<0.1	<0.1	<0.1
Cyproterone acetate	90	n.d.	n.d.	n.d.
Medroxyprogesterone acetate	115	n.d.	n.d.	n.d.

n.d., Not determined.

Table VI. Relative binding affinities (%) of different progestogens for binding to human uterine estrogen receptor, rat liver glucocorticoid receptor, rat kidney mineralocorticoid receptor, and rat prostate androgen receptor

	Gludocorticoid receptor	*Mineralocorticoid receptor*	*Androgen receptor*	*Estrogen receptor*
	Dexamethasone	*Aldosterone*	*R1881*	*Estradiol*
Progesterone	10	100	<0.1	<0.1
Gestodene	27	350	100	<0.1
Levonorgestrel	0.6	70	45	<0.1
R5020	17	53	0.1	<0.1
Org2058	3	27	<0.1	<0.1
Desogestrel	<0.1	<0.1	<0.1	<0.1
3-Ketodesogestrel	14	<0.1	20	<0.1
Norgestimate	<0.1	<0.1	<0.1	<0.1
Cyproterone acetate	6	8	6	<0.1
Medroxyprogesterone acetate	29	160	5	<0.1

steroid most frequently used in receptor studies. When employing R5020, it is shown that the progestogens such as cyproterone acetate, gestodene, and levonorgestrel (which are frequently used in oral contraceptives) and the biologically active metabolite 3-ketodesogestrel (which is only formed from the prodrug desogestrel in the body) bind to the progesterone receptor with high affinity apart from slight differences. The RBA values are markedly higher than those of progesterone, the natural gestagen. No measurable binding affinity at the progesterone receptor could be demonstrated for norgestimate in the system used, indicating that this substance (similar to desogestrel) constitutes a prodrug that must be transformed into the biologically active form by metabolic processes in the body.

If tritium-labeled gestodene, 3-ketodesogestrel, or levonorgestrel are used as reference steroids, a different arrangement is seen in the hierarchy of the RBA values in some cases (Table V and Fig. 4). For example, R5020 is only a weak competitor when compared with the reference steroids mentioned. Thus, 3-ketodesogestrel is able to displace gestodene from its binding sites in the uterine cytosol only with a reduced binding affinity, whereas gestodene conversely proves to be a high-affinity competitor for tritiated 3-ketodesogestrel.

Gestodene, 3-ketodesogestrel, and medroxyprogesterone acetate bind with relatively high affinity for the glucocorticoid receptor from rat livers of adrenalectomized animals comparable to that of progesterone, whereas cyproterone acetate, and to a greater extent levonorgestrel, only interact with lower affinity with the binding area of the glucocorticoid receptor (Table VI and Fig. 5).

The high affinity of gestodene when compared with that of the mineralocorticoid receptor from kidney cytosol of adrenalectomized rats is striking (Table VI and Fig. 5). In relation to aldosterone, the RBA value is 350%, which indicates a strong mineralocorticoid or antimineralocorticoid action of this compound at the receptor level. Medroxyprogesterone acetate, levonorgestrel, and progesterone, the natural gestagen itself, also have high binding affinities to the mineralocorticoid receptor, whereas 3-ketodesogestrel and norgestimate do not display any measurable affinity for this receptor species.

Furthermore, gestodene, levonorgestrel, and 3-ketodesogestrel bind with relatively high affinity to the

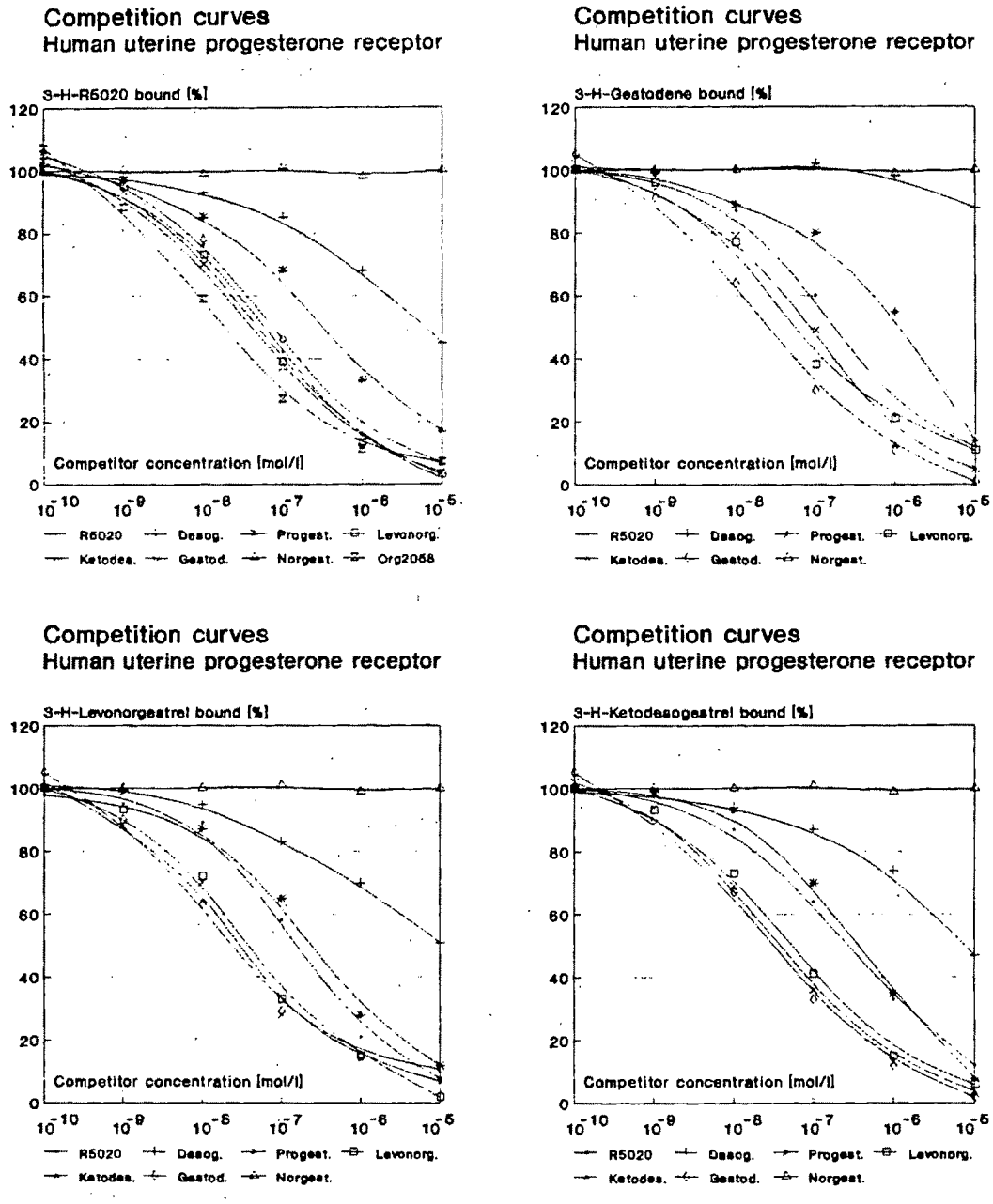


Fig. 4. Specificity of the binding of different tritium-labeled progestins to the cytoplasmic progesterone receptor in human uterus.

androgen receptor from prostate cytosol of rats but to a lesser extent (Table VI and Fig. 5). Cytoproterone acetate acts as a highly effective antiandrogen in addition to its partial gestagen effect. Astonishingly, it displays an RBA value of only 6% compared with the synthetic ligand R1881 (functioning as reference steroid [=100%]).

Estrogen receptor affinity could not be demonstrated in any of the progestogens investigated (Table VI and Fig. 5).

Sucrose density gradient centrifugation. Fig. 6 shows density gradient profiles of human uterine cytosol, which was incubated with different tritium-labeled synthetic progestogens. Tritium-labeled progesterone receptor complexes that settled as sediment in the 8S and 4S region of the linear sucrose gradient can be demonstrated for all ligands tested. In contrast to Org2058, a highly potent synthetic ligand for progesterone receptor analysis, it is evident that when gestodene, levonorgestrel, or 3-ketodesogestrel are used as ligands, a high proportion of tritium binding in the 4S region is to be observed. The ligand cannot be displaced, even with high molar excess of authentic steroid or Org2058.

This is to be rated as manifestation of relatively high

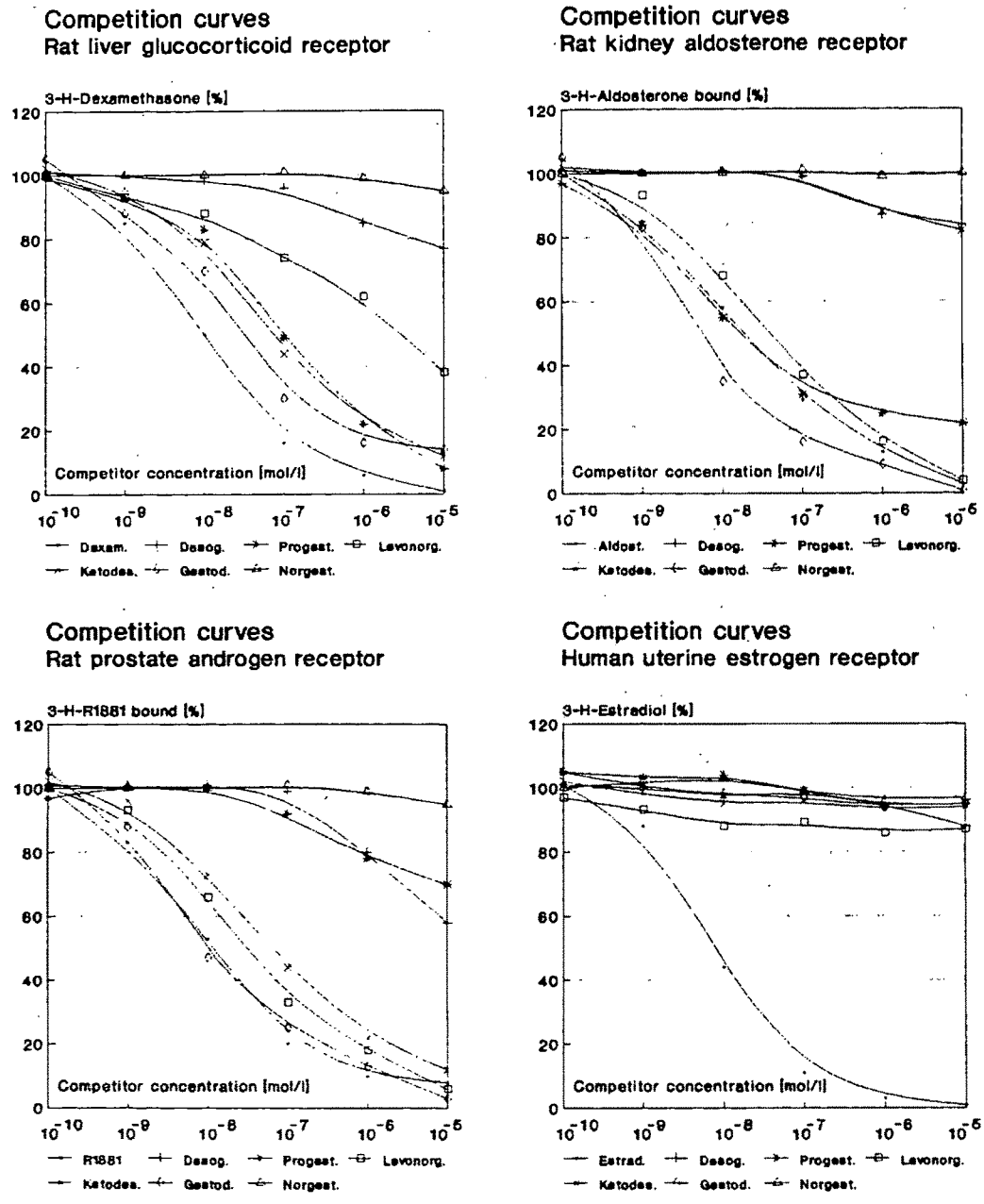


Fig. 5. Specificity of the binding of different tritium-labeled steroids to the cytoplasmic glucocorticoid receptor of rat liver, mineralocorticoid receptor of rat kidney, androgen receptor of rat prostate, and estrogen receptor of human uterus.

nonspecific binding activity of these synthetic ligands for uterine cytosol.

Comment

In terms of present day understanding, it is generally accepted that a correlation seems to exist between receptor binding of steroid hormones and biologic activity of these compounds in vivo. In this connection, however, it is to be mentioned that competitive analysis, with which the binding affinities are measured as compared with a reference steroid as RBA values, is doubtless incapable of establishing whether high binding affinity acts agonistically or antagonistically at the receptor level. In vivo experiments will be required to establish this characteristic.

In the Clauberg test, the standard method of determining gestagenic activity—because it detects the capacity of the progestogen to transform the estrogen-stimulated endometrium—gestodene is superior to all known progestogens including 3-ketodesogestrel.[12] For example, gestodene is three times more potent than levonorgestrel. However, this does not reflect the sit-

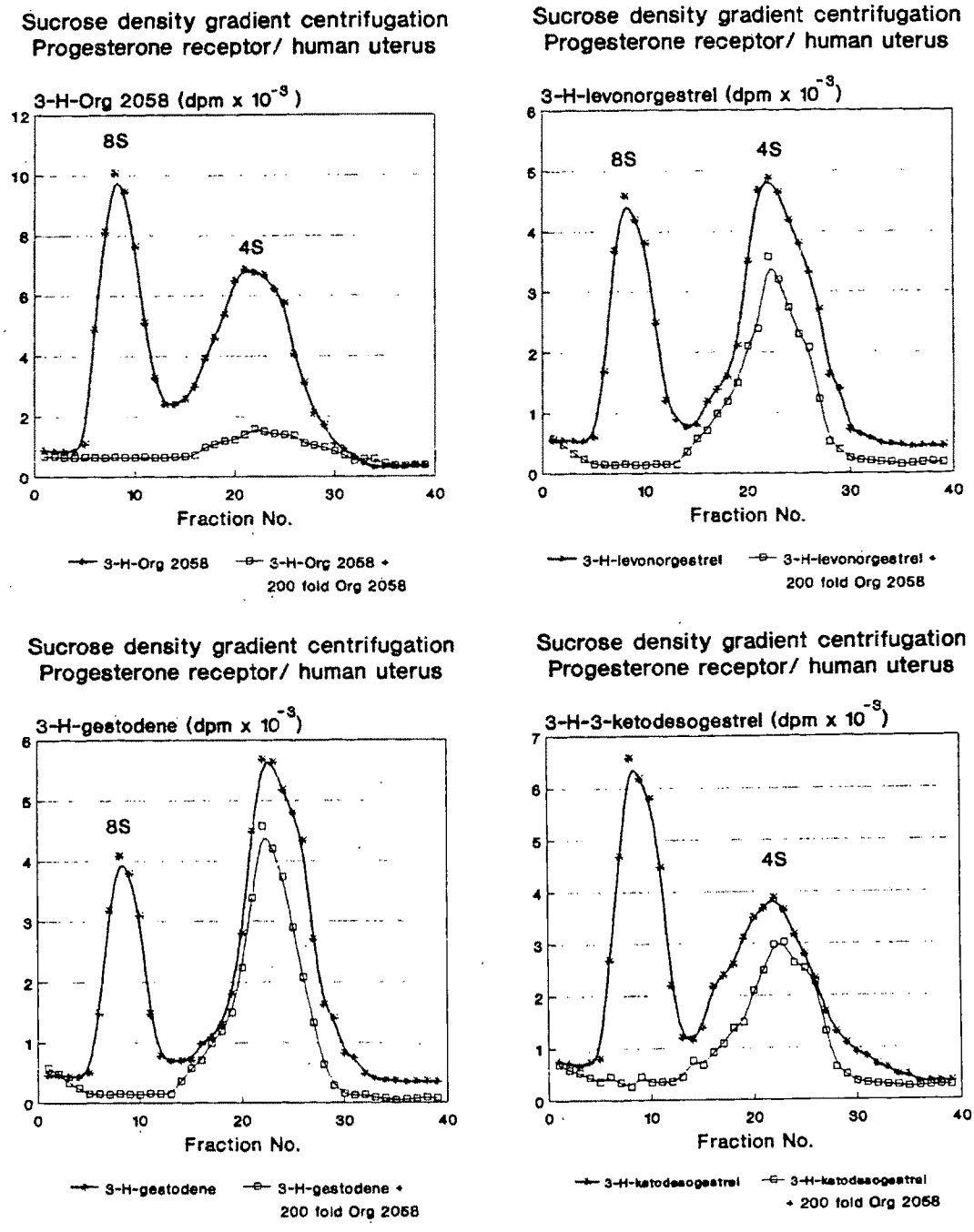


Fig. 6. Sucrose density gradient centrifugation of human myometrial cytosol preincubated with different tritium-labeled synthetic progestins.

uation with regard to the affinity to the progesterone receptor. In this connection it should be mentioned that in vitro binding affinities were determined at 4° C, whereas clinically relevant receptor binding occurs at 37° C.

An important aspect of the endocrinologic profile of progestogens of the gonane series is their androgenic activity. It is surprising to find that gestodene, despite its distinctly more powerful binding affinity to the androgen receptor, does not possess correspondingly more potent androgenicity than levonorgestrel or 3-ketodesogestrel (Herschberg test).[12]

Attention should be drawn to the fact that gestodene has a surprisingly high affinity for the aldosterone receptor. This affinity corresponds to weak antialdosterone activity in adrenalectomized rats,[12, 20] in which gestodene partially blocks the elimination of sodium with the urine. Whether this can have any clinical significance is doubtful, because significantly higher dosages of gestodene are needed for the antialdosterone activity

in comparison to the low level that exerts contraceptive efficacy in women. In this respect, gestodene can be compared with progesterone which, in physiologic doses, has a natriuretic effect in humans.[21-24] In contrast, 3-ketodesogestrel is characterized by a lack of binding to the mineralocorticoid receptor of kidney tissue of adrenalectomized rats. Furthermore, the competition studies demonstrated that desogestrel (but also norgestimate) function as prodrugs because the pattern of binding at the hormone receptors show no affinity.

As anticipated, sucrose gradient centrifugation analysis of cytosol of human myometrial tissue after saturation with tritium-labeled R5020, gestodene, cyproterone acetate, levonorgestrel, and 3-ketodesogestrel showed that the progestogen-binding components sedimented in the 4S and 8S region. In use of tritiated R5020 as binding partner, a 200-fold molar excess of nonradioactive R5020 almost led to complete reduction of the radioactivity, both in the 8S and in the 4S region. On the other hand, only a reduction of the 8S binding is to be observed in use of the other synthetic tritium-labeled progestogens in the presence of a 200-fold molar excess of nonradioactive authentic progestogen itself or R5020 as competitors. Binding of 4S was not reduced, which indicates the existence of a second high-capacity binding component. The nature of this binder is speculative. Because a 200-fold molar excess of nonradioactive DHT also does not lead to any appreciable reduction in protein-bound radioactivity in the 4S region, SHB is to be largely ruled out as binder.

The nonspecific binding in the 4S binding area visualized by sucrose density gradient analysis is reflected in the results obtained by HPLC analysis. Here, binding peaks are also to be observed that are not imaged in use of tritiated Org2058, or are only restricted to a limited extent in the presence of a 200-fold molar excess of nonradioactive Org2058 in the remaining spectrum of tritium-labeled progestogens.

As mentioned in the beginning, the biologic activity of a synthetic as well as a natural steroid hormone is not determined only by the binding properties on the receptor level; in addition, the distribution of steroid hormones between various protein-bound and nonprotein-bound components of the serum is of crucial importance for the bioavailability in different target tissues.

The binding of several contraceptive steroids to SHBG has been studied in detail,[25-32] and some information is available on the relative binding of ethinylestradiol, norethisterone, and levonorgestrel to serum albumin, but little information is available about how changes in circulating concentrations of steroid-binding proteins influence the plasma distribution of endogenous or contraceptive steroids.

In several publications,[31-33] it is reported that oral contraceptives that contain gestodene, but also 3-ketodesogestrel (much used progestogens in low-dose oral contraceptives of the most recent generation), lead to an increase of SHBG and CBG blood levels in consequence of increased hepatic synthesis of these proteins induced by high concentrations of ethinylestradiol in the hepatic-portal circulation. With regard to levonorgestrel, it is of interest to note that the same effect was not seen for SHBG. Levonorgestrel suppresses the ethinylestradiol-induced increase in serum SHBG as a function of the ratio of the levonorgestrel/ethinylestradiol doses in the pill, but its mechanism of action is not known. With regard to CBG, levonorgestrel (similar to gestodene and 3-ketodesogestrel) also leads to an increase of the serum concentration similar to that observed in women given ethinylestradiol alone.

Recently, in a comparison study with two low-dose oral contraceptives (one preparation contained 150 μg desogestrel and 30 μm ethinyl estradiol and the other preparation contained 75 μg gestodene as gestagenic component), Kuhl et al.[33-35] were able to show the following:

1. Serum levels of 3-ketodesogestrel, the biologically active metabolite of desogestrel, and gestodene rose in the course of an administration cycle.
2. Up to the third cycle of administration, the gestodene concentration revealed identical time courses in each cycle of administration. After 1 year, a fall in concentration was measured for 3-ketodesogestrel alone.
3. The absolute concentrations of gestodene and the rises within a cycle and over the first three cycles were higher than with 3-ketodesogestrel.
4. For gestodene, an unequivocal dependence of the serum levels on the concentration of SHBG was demonstrated. This was not the case for 3-ketodesogestrel.

One objective of the present study was to characterize the binding properties on the level of serum proteins of numerous synthetic progestins used in oral contraceptives. Summarizing the results, the following observations can be made: compared with levonorgestrel and gestodene, 3-ketodesogestrel reveals a relative binding activity with regard to SHBG that is one order of magnitude lower. This finding tends to corroborate the results of other authors.[25-33] Furthermore, marked differences in the binding kinetics between these synthetic progestogens are striking. Gestodene and levonorgestrel both form long-lived steroid protein complexes with SHBG, whereas the SHBG complexes formed with 3-ketodesogestrel are characterized by a short half-life.

If the results of Kuhl and associates,[33-35] with regard to higher gestodene blood levels with half the dosage when compared with 3-ketodesogestrel, are evaluated

in the light of this data, it can be observed that the results presented (in combination with the observation of the elevation of SHBG blood levels under oral contraceptives containing gestodene or desogestrel made by numerous authors[25, 33-35]) are sufficient to explain this phenomenon of the increase of gestodene over the administration cycles by higher affinity of binding to steroid-binding serum proteins as well as formation of stable gestodene-SHBG complexes. Any other explanation—for example, the delayed enzymatic inactivation of gestodene by the inhibitory effect of this steroid on the microsomal P_{450} enzyme system of the liver postulated for example by Kuhl—is speculative and must first be validated by unequivocal experimental data.

REFERENCES

1. Djerassi C, Miramontes L, Rosenkranz G, Sondheimer F. Steroids LIV. Synthesis of 19-Nor-17α-ethinyltestosterone and 19-Nor-17α-methyl-testosterone. J Am Chem Soc 1954;76:4472-9.
2. Jost A III. Role des gonades foetales dans la differenciation sexualle somatique. Arch Anat Microsc Morphol Exp 1946;36:271-315.
3. Elger W. Die rolle der fetalen androgene in der sexualdifferenzierung des kaninchens und ihre abgrenzung gegen andere hormonale und somatische faktoren durch anwendung eines starken antiandrogens. Arch Anat Microsc Morphol Exp 1966;55:657-743.
4. Gaspard UJ. Role of antiandrogens in the treatment of acne. In: Mahesh VR, Greenblatt RB, eds. London: John Wright–PSG Inc., 1983:369-94.
5. Hammerstein J, Lachnit-Fixson U, Neumann F, Plewig G, eds. Androgenization in women. Acne, seborrhoe, androgenetic alopecia and hirsutism. Amsterdam, Excerpta Medica, 1980:1-298.
6. Viinikka L. Metabolism of a new synthetic progestagen, Org2969, by human liver in vitro. J Steroid Biochem 1978;10:353-7.
7. Viinikka L, Ylikorkala O, Vihko R, et al. Metabolism of a new synthetic progestagen, Org 2969, in female volunteers. Pharmacokinetics after an oral dose. Eur J Clin Pharmacol 1979;15:349-54.
8. Bergink EW, Bos ES, Kloosterboer HJ, Lund L, Nummi S, Van Der Vies J. Comparison of the metabolic effects of desogestrel and of levonorgestrel in low-dose oestrogen oral contraceptives. In: van Keep PE, Kopera H, eds. Oral contraceptives and lipoproteins. Brussels. International Health Foundation, 1983:52-64.
9. Bergink EW, Hamburger AD, De Jager E, Van Der Vies J. Binding of a contraceptive progestogen, Org 2969, and its metabolites to receptor proteins and human sex hormone binding globulins. J Steroid Biochem 1981;14:175-83.
10. Hofmeister H, Wiechert R, Annen K, Laurent H, Steinbeck H. DE 25.46.062/10 Okt. Chemical Abstracts 87, 168265k/1977, 1975.
11. Hofmeister H, Annen K, Laurent H, Petzoldt K, Wiechert R. Synthesen von gestoden. Drug Research 1986;36:781.
12. Beier S, Steinbeck H, Schillinger E, Losert W, Elger W. Endocrinological studies with gestodene—a new progestogen. In: Genazzani AR, Volpe A, Facchinetti F, eds. Research reports on gynecology and obstetrics. Park Ridge, New Jersey: Parthenon Publishing Group, 1986:477-83.
13. Raabe T, Runnebaum B, Grunwald K, et al. Metabolic effects of a gestodene containing low dose pill. In: Breckwoldt M, Düsterberg B, eds. Gestodene, a new direction oral contraception. New Jersey: Parthenon Publishing Group, 1989:31-45.
14. Hahn DW, Allen GO, McGuire JL. The pharmacological profile of norgestimate, a new orally active progestin. Contraception 1977;16:541-9.
15. Lowry OH, Rosebrough NF, Farr AG, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
16. Korenman SG. Relation between estrogen inhibitory activity and binding to cytosol of rabbit and human uterus. Endocrinology 1970;87:1119-23.
17. Scatchard G. The attraction of proteins for small molecules and ions. Ann NY Acad Sci 1949;51:660-72.
18. Heubner A, Rüde S, Juchem M, Grill HJ, Pollow K. An enzyme-linked immunosorbent assay (ELISA) for human sex hormone-binding globulin. J Steroid Biochem 1987; 28:647-52.
19. Heubner A, Belovsky O, Müller W, Grill HJ, Manz B, Juchem M, Pollow K. Application of liquid partition chromatography in the purification of sex hormone binding globulin. J Chromatography 1987;397:419-34.
20. Losert W, Casals-Stenzel J, Buse M. Progestogens with antimineralocorticoid activity. Drug Res 1985;53:459-67.
21. Landau RL, Bergenstal DM, Lugibihl K, Kascht ME. The metabolic effects of progesterone in man. J Clin Endocrinol Metab 1955;15:1194-9.
22. Landau RL, Lugibihl K. Inhibition of the sodium-retaining influence of aldosterone by progesterone. J Clin Endocrinol Metab 1958;18:1237-45.
23. Landau RL, Lugibihl K. The catabolic and natriuretic effects of progesterone in man. Recent Prog Horm Res 1961;17:249-62.
24. Wambach G, Higgins JR. Antimineralocorticoid action of progesterone. In: Agarval MK, ed. Antihormones. Amsterdam: Elsevier North-Holland Biomedical Press, 1979: 167-79.
25. Hammond GL, Langley MS, Robinson PA, Lund L. Serum steroid binding protein concentrations, distribution of progestogens, and bioavailability of testosterone during treatment with contraceptives containing desogestrel or levonorgestrel. Fertil Steril 1984;42:44-51.
26. Sitteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW. The serum transport of steroid hormones. Recent Prog Horm Res 1982;38:457-510.
27. Van Kammen E, Thijssen JHH, Rademaker B, Schwarz F. The influence of hormonal contraceptives on sex hormone binding globulin (SHBG) capacity. Contraception 1975;11:53-9.
28. Hammond GL, Lähteenmäki PLA, Lähteenmäki P, Luukkainen I. Distribution and percentages of non-protein bound contraceptive steroids in human serum. J Steroid Biochem 1982;17:375-80.
29. Schwartz U, Hammerstein J. The oestrogenic potency of various contraceptive steroids as determined by their effects on transcortin binding capacity. Acta Endocrinol (Copenh) 1974;76:159-71.
30. Victor A, Johansson EDB. Effects of D-norgestrel induced decreases in sex hormone binding globulin capacity on the D-norgestrel levels in plasma. Contraception 1977; 16:115.
31. El Makhzangy MN, Wynn V, Lawrence DM. Sex hormone binding globulin capacity as an index of oestrogenicity or androgenicity in women on oral contraceptive steroids. Clin Endocrinol (Oxf) 1981;10:39.
32. Bergink EW, Holma P, Pyörälä T. Effects of oral contraceptive combinations containing levonorgestrel or desogestrel on serum proteins and androgen binding. Scand J Clin Lab Invest 1981;41:663-8.

33. Jung-Hoffmann C, Heidt F, Kuhl H. Effect of two contraceptives containing 30 μg ethinylestradiol and 75 μg gestodene or 150 μg desogestrel upon various hormonal parameters. Contraception 1988;38:593-603.
34. Kuhl H, Jung-Hoffmann C, Heidt F. Serum levels of 3-ketodesogestrel and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 150 μg desogestrel. Contraception 1988;38:381-90.
35. Kuhl H, Jung-Hoffmann C, Heidt F. Alterations in the serum levels of gestodene and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 75 μg gestodene. Contraception 1988;38:477-86.

Pharmacokinetics and pharmacodynamics of oral contraceptive steroids: Factors influencing steroid metabolism

Claudia Jung-Hoffmann, MD, and Prof. Dr. Herbert Kuhl, PhD
Frankfurt am Main, Federal Republic of Germany

The time-dependent alterations in the serum concentrations of ethinyl estradiol, gestodene, and 3-keto-desogestrel during treatment with 30 μg of ethinyl estradiol + 75 μg of gestodene or 30 μg of ethinyl estradiol + 150 μg of desogestrel were investigated during 12 months. The levels of gestodene and 3-keto-desogestrel increased between days 1 and 21 of each cycle, reaching maximal levels during the third and sixth cycles. The serum concentrations of gestodene were fourfold to fivefold higher than those of 3-keto-desogestrel. The ethinyl estradiol levels increased significantly between days 1 and 10 during each cycle and were significantly higher by 70% during intake of ethinyl estradiol/gestodene compared with ethinyl estradiol/desogestrel, although the dose was identical. Intake of gestodene, in addition to 35 μg of ethinyl estradiol + 2 mg of cyproterone acetate, caused a rise in ethinyl estradiol levels. During treatment with ethinyl estradiol/gestodene and an additional 150 μg of levonorgestrel, there was a continuous increase in gestodene levels, although sex hormone–binding globulin level did not change. During treatment with 30 or 35 μg of ethinyl estradiol and 75 μg of gestodene, 150 μg of desogestrel, or 2 mg of cyproterone acetate, there were large intraindividual and interindividual variations in the steroid levels and ratios of estrogen:progestogen levels. There was no correlation with the occurrence of intermenstrual bleedings. It is concluded that ethinyl estradiol and nortestosterone derivatives may inhibit steroid-metabolizing enzymes in the liver, which results in a rise in the serum levels of contraceptive steroids. The cause of the large intraindividual variations is as yet unknown, but it is probably from changes in steroid metabolism. (Am J Obstet Gynecol 1990;163:2183-97.)

Key words: Pharmacokinetics of contraceptive steroids, ethinyl estradiol, gestodene, 3-keto-desogestrel

Drug monitoring is considered as an approach to minimize adverse reactions and side effects by individual adjustment of the dose. Although the susceptibility of women to a given serum level may vary, the average biologic effects are assumed to be related to the serum concentration rather than to the dose ingested.

Because the pill must suppress ovulation reliably in all women, it is overdosed in many women. Even though contraceptive steroids have a large therapeutic margin, they may cause severe adverse effects. There are reports on elevated serum concentrations of ethinyl estradiol in women who developed hypertension during treatment with the pill.[1,2] Little information is available on correlations between contraceptive steroid levels and metabolic parameters. A significant inverse correlation has been observed between the serum levels of medroxyprogesterone acetate and high-density lipoprotein cholesterol.[3] No relationship was found between the serum levels of ethinyl estradiol or norgestrel and plasma Xa inhibitory activities.[4] Because of the large interindividual and intraindividual variations of the steroid concentrations, even after intravenous and vaginal application,[5-7] it will be difficult to recognize such correlations when only single blood samples were taken. Moreover, many of the metabolic parameters are

From the Division of Gynecological Endocrinology, Department of Obstetrics and Gynecology, J. W. Goethe University.
Reprint requests: Prof. Dr. Herbert Kuhl, Division of Gynecological Endocrinology, Department of Obstetrics and Gynecology, J. W. Goethe University, Theodor-Stern-Kai 7, D-6000 Frankfurt am Main 70, Federal Republic of Germany.
6/0/23264

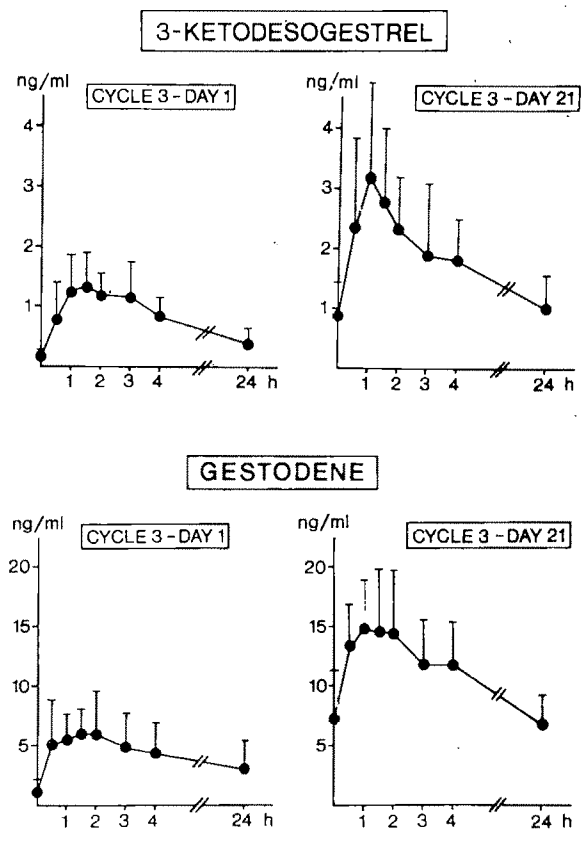


Fig. 1. Serum levels of 3-keto-desogestrel and gestodene on days 1 and 21 of the third cycle of treatment with 30 μg of ethinyl estradiol + 150 μg desogestrel or 30 μg of ethinyl estradiol + 75 μg of gestodene.

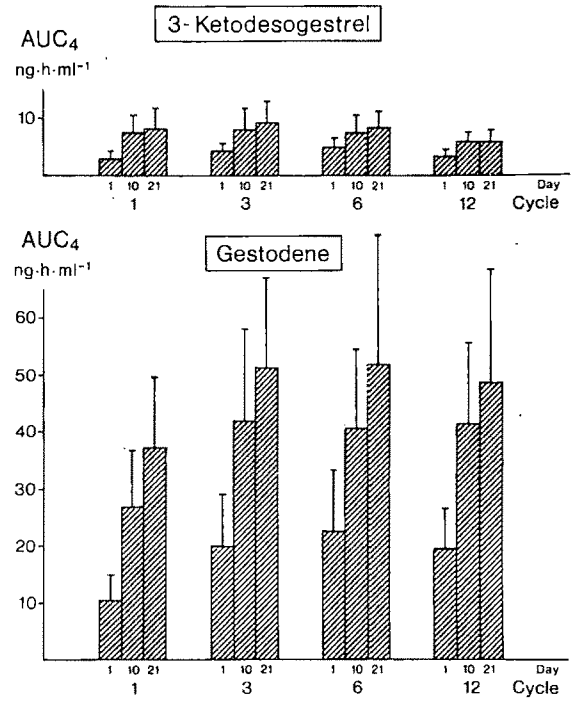


Fig. 2. Areas under the time versus serum concentration curves between 0 and 4 hours of 3-ketodesogestrel and gestodene during treatment with 30 μg of ethinyl estradiol + 150 μg of desogestrel or 30 μg of ethinyl estradiol + 75 μg of gestodene.

influenced by the estrogenic and progestogenic components in an antagonistic manner and may change with time of treatment. The alterations in hepatic metabolism during and after treatment with oral contraceptives take place with a lag time of several days or weeks. Therefore metabolic serum parameters may reflect an integral effect of average steroid levels rather than day-to-day variations. The measurement of steroid levels in single blood samples and even the determination of the concentration versus time curves on a single day represent more or less a snapshot that may differ from the long-term status of an individual woman.

In the present studies the individual serum concentrations of ethinyl estradiol and different progestogens during treatment with low-dose oral contraceptives were measured during the first 4 hours after intake of the pill and 24 hours after that on several days.

Material and methods

Study 1. Twenty-two normal healthy volunteers with regular menstrual cycles, between 18 and 35 years old, participated in the study. Informed consent was obtained. The women had not used hormonal contraceptives for at least 3 months before the study and were not under pharmacologic therapy. An internal and gynecologic examination, including a Papanicolaou smear, was carried out before the selection of the subjects. During a control cycle, ovulation was confirmed to have probably taken place by the determination of serum progesterone (>3 ng/ml).

Two groups of 11 women each were randomly assigned to take a combination of 30 μg of ethinyl estradiol and 75 μg of gestodene (Femovan, Schering AG, Berlin) or a combination of 30 μg of ethinyl estradiol and 150 μg of desogestrel (Marvelon, Organon GmbH, Oberschleissheim) during a 12-month period. Blood samples during fasting were obtained between 8:00 and 10:00 AM on days 1, 10, and 21 of the first, third, sixth, and twelfth treatment cycles immediately before and 0.5, 1, 1.5, 2, 3, 4, and 24 hours after ingestion of a tablet. After centrifugation, the serum was stored at −20° C until analysis.

Study 2. Twelve normal healthy volunteers with regular menstrual cycles, between the ages of 25 and 43 years, participated in the study. Informed consent was obtained. Except for volunteer No. 9 who took 150 μg of thyroxine daily, the women were not under pharmacologic therapy. An internal and gynecologic examination was carried out before the start of the study. The women were treated with a combination of 35 μg of ethinyl estradiol and 2 mg of cyproterone acetate (Diane 35, Schering AG, Berlin).

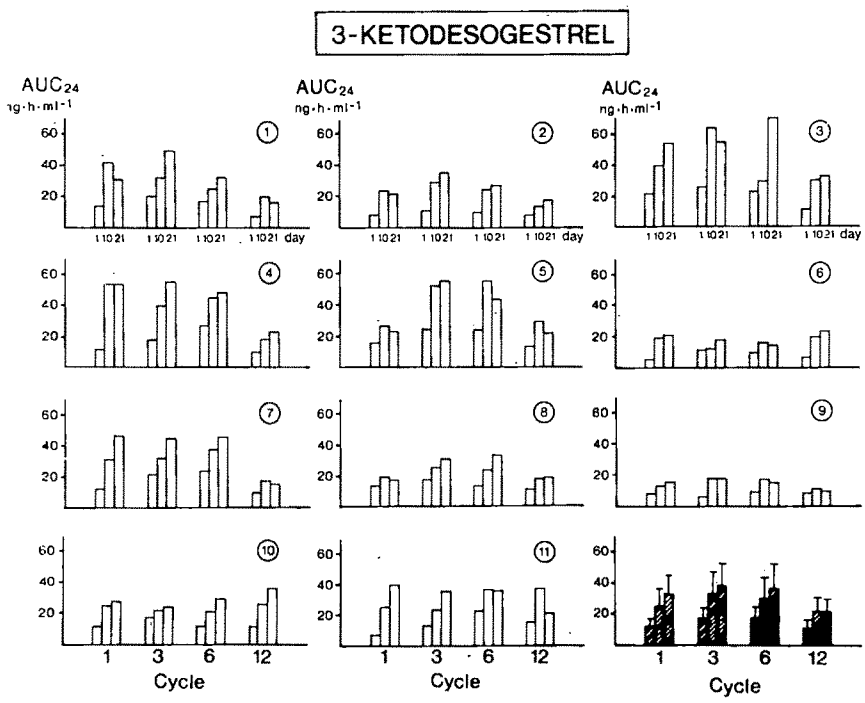


Fig. 3. Individual areas under the time versus serum concentration curves of 3-keto-desogestrel in 11 volunteers during 12 months of treatment with 30 μg of ethinyl estradiol + 150 μg of desogestrel *(hatched columns:* Mean ± SD).

Blood samples during fasting were taken between 8:00 and 10:00 AM on days 1, 10, and 21 of one cycle immediately before and 0.5, 1, 1.5, 2, 3, 4, and 24 hours after ingestion of a tablet. After centrifugation, the serum was stored at −20° C until analysis.

Study 3. One woman (one of the authors) ingested a combination of 35 μg of ethinyl estradiol and 2 mg of cyproterone acetate for 26 days. In addition, she took 150 μg of gestodene on days 6, 13, and 20, and 75 μg of gestodene on days 7, 8, 14, 15, 21, and 22. Blood samples during fasting were obtained between 8:00 and 10:00 AM on days 1, 5, 8, 12, 15, 19, 22, and 26 immediately before and 0.5, 1, 1.5, 2, 3, 4, and 24 hours after intake of the tablets. After centrifugation, the serum was stored at −20° C until analysis.

Study 4. The same woman switched from treatment with a combination of 35 μg of ethinyl estradiol and 2 mg of cyproterone acetate after a pill-free week to a combination of 30 μg of ethinyl estradiol and 75 μg of gestodene for one cycle, taking an additional 150 μg of levonorgestrel daily. After a hormone-free interval of 7 days, she took 30 μg of ethinyl estradiol and 75 μg of gestodene for one cycle. Thereafter, she ingested 150 μg of levonorgestrel daily during the interval of 7 days, and after that she started one cycle of treatment with 30 μg of ethinyl estradiol and 75 μg of gestodene and an additional 150 μg of levonorgestrel. Blood samples during fasting were taken between 8:00 and 10:00 AM on days 1, 10, and 21 of the cycle immediately before and 0.5, 1, 1.5, 2, 3, 4, and 24 hours after intake of a tablet. After centrifugation, the serum was stored at −20° C until analysis.

Laboratory methods. The serum concentrations of ethinyl estradiol were measured in duplicate by means of radioimmunoassay after extraction of 1 ml of serum with diethylether. The method has been described elsewhere in detail.[8] The serum concentrations of 3-keto-desogestrel, which is the biologically active metabolite of desogestrel, were determined in duplicate by means of radioimmunoassay after chromatographic fractionation. The method has been described elsewhere in detail.[9] The serum concentrations of gestodene were measured in duplicate by means of radioimmunoassay after extraction of 0.1 ml of serum with diethylether. The method has been described elsewhere in detail.[10]

Serum concentrations of sex hormone–binding globulin (SHBG) were determined by means of a "liquid phase" immunoradiometric assay kit (Farmos Diagnostica, Oulunsalo, Finland).

Statistical analysis. The areas under the serum concentration versus time curves between 0 and 24 hours were calculated by the trapezoidal rule. Data were statistically analyzed by multivariate analysis of variance (repeated measurements design) or by the Student paired *t* test.

Results

During each cycle of treatment with ethinyl estradiol/desogestrel or ethinyl estradiol/gestodene, the

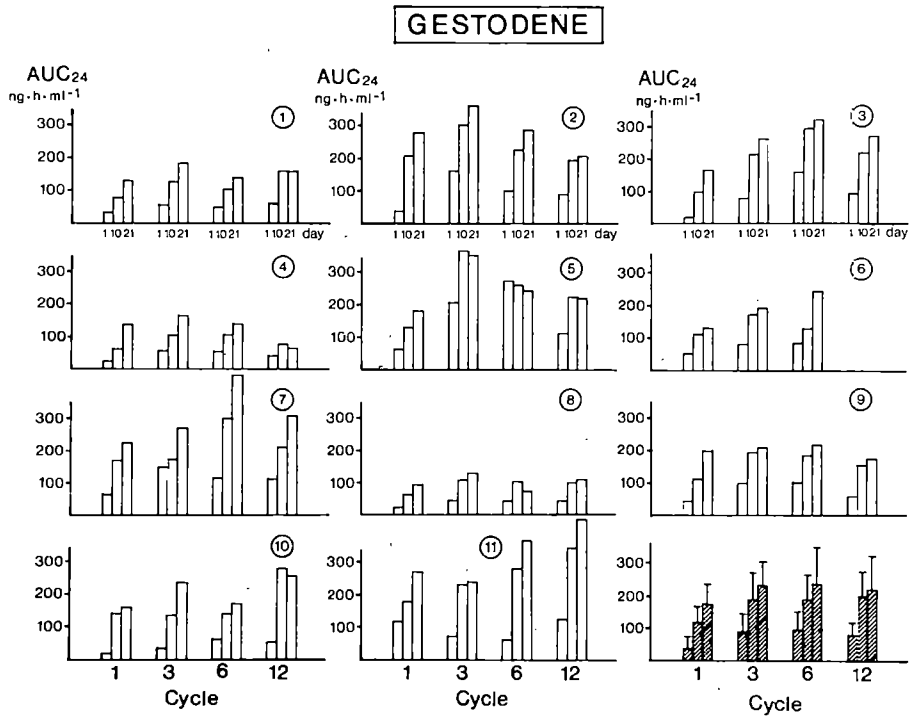


Fig. 4. Individual areas under the time versus serum concentration curves of gestodene in 11 volunteers during 12 months of treatment with 30 μg of ethinyl estradiol + 75 μg of gestodene (*hatched columns:* Mean ± SD).

serum concentrations of 3-keto-desogestrel and gestodene increased significantly from day 1, reaching maximal levels between days 10 and 21. During the third and sixth cycles, progestogen levels were significantly higher than during the first cycle (Fig. 1). Thereafter no further increase was observed. The 3-keto-desogestrel levels during the twelfth cycle were even significantly lower than before.[9, 10] When the areas under the curves (AUCs) between 0 and 4 hours (Fig. 2), as well as between 0 and 24 hours were compared, it was conspicuous that the levels of gestodene were fourfold to fivefold those of 3-keto-desogestrel, although the dose of gestodene was only half of that of desogestrel.[9, 10]

There were large intraindividual and interindividual variations both in the 3-keto-desogestrel and gestodene levels as demonstrated in the AUCs between 0 and 24 hours measured on days 1, 10, and 21 of the first, third, sixth, and twelfth cycles in all volunteers taking ethinyl estradiol/desogestrel (Fig. 3) or ethinyl estradiol/gestodene (Fig. 4). The average intraindividual and interindividual coefficients of variation were in the range between 30% and 70%, but were less pronounced in most women when the increases in steroid levels during each cycle were regarded as a rule. Some women showed permanently low 3-keto-desogestrel or gestodene levels, whereas others remained at relatively high progestogen concentrations throughout the 12 treatment cycles (Figs. 3 and 4).

When the serum concentrations of ethinyl estradiol were determined, the results were quite surprising. Although the ethinyl estradiol dose of 30 μg was identical in both preparations, ethinyl estradiol levels were significantly higher during intake of the gestodene-containing pill (Figs. 5 and 6). The calculation of the AUCs between 0 and 4 hours revealed a difference of 37% and between 0 and 24 hours 70% on average.[8] There was a close correlation between the AUCs 0 to 4 hours and the AUCs 0 to 24 hours. It is also remarkable that after intake of ethinyl estradiol/gestodene, the ethinyl estradiol peaks occurred 30 minutes to 1 hour later than with ethinyl estradiol/desogestrel. During each cycle of treatment with both preparations, there was a significant rise in ethinyl estradiol levels between days 1 and 10 that remained thereafter at this level, indicating that a steady state was reached between days 1 and 10 (Figs. 7 and 8; *hatched columns*). In contrast to the progestogen concentrations, there was no difference between the ethinyl estradiol levels of the first and those of subsequent cycles. Similar to the progestogen levels, there were large intraindividual and interindividual variations in the ethinyl estradiol concentrations. In some women, however, we found low estrogen levels on each of the 12 days investigated,

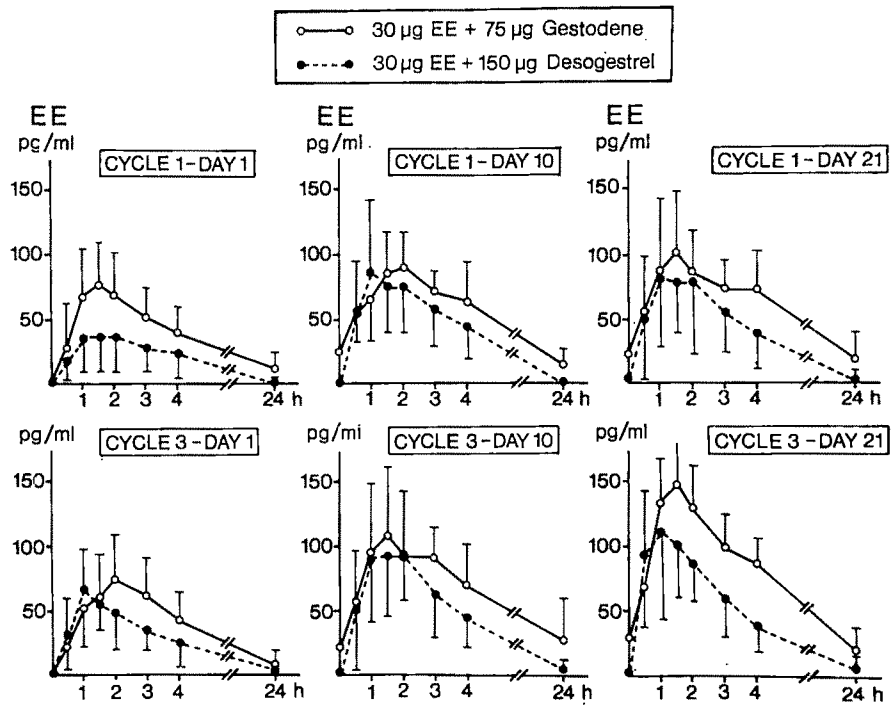


Fig. 5. Serum concentrations of ethinyl estradiol *(EE)* during 24 hours after intake of a tablet of ethinyl estradiol/gestodene or ethinyl estradiol/desogestrel on days 1, 10, and 21 of the first and third treatment cycles (mean ± SD). (Reprinted from Jung-Hoffmann C, Kuhl H. Contraception 1989;40:299-312, by permission of Butterworth-Heinemann [U.S.].)

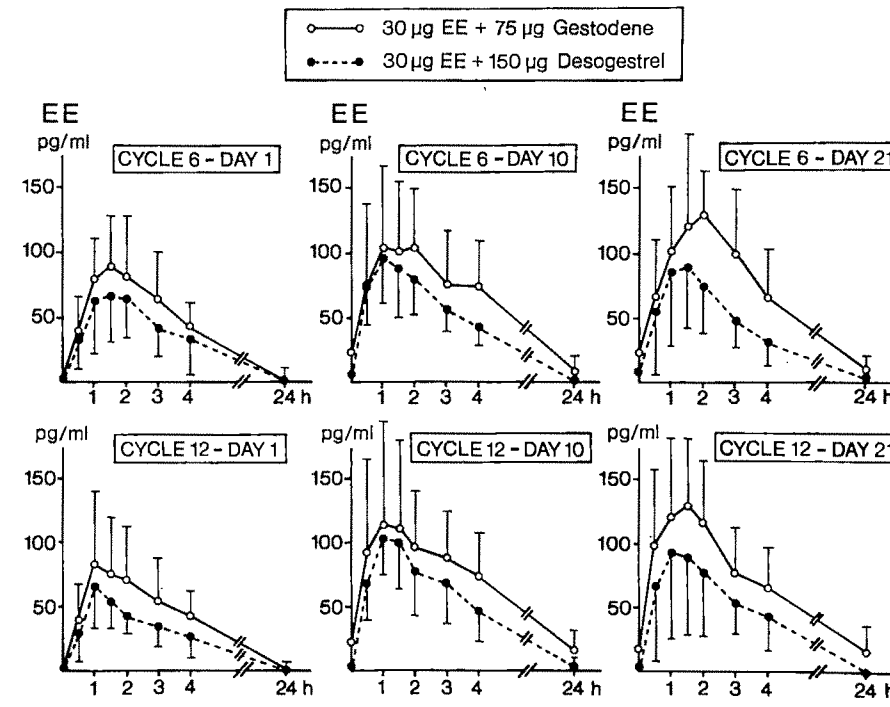


Fig. 6. Serum concentrations of ethinyl estradiol during 24 hours after intake of a tablet of ethinyl estradiol/gestodene or ethinyl estradiol/desogestrel on days 1, 10, and 21 of the sixth and twelfth treatment cycles (mean ± SD). (Reprinted from Jung-Hoffmann C, Kuhl H. Contraception 1989; 40:299-312, by permission of Butterworth-Heinemann [U.S.].)

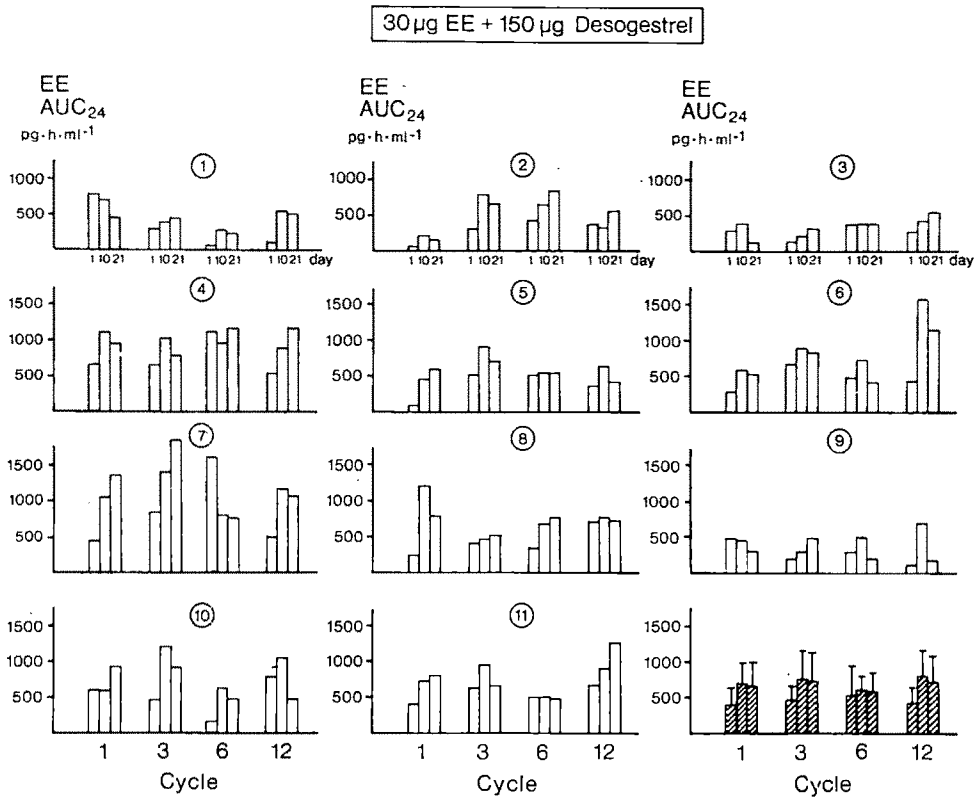


Fig. 7. Individual areas under the time versus serum concentration curves of ethinyl estradiol in 11 volunteers during 12 months of treatment with 30 µg of ethinyl estradiol + 150 µg of desogestrel (*hatched columns:* Mean ± SD).

whereas some other volunteers showed permanently high concentrations. When the individual ratios between the serum concentrations of ethinyl estradiol and 3-keto-desogestrel or gestodene were calculated, large intraindividual and interindividual variations were found (Figs. 9 and 10). As represented by the hatched columns, the average values show that in each cycle there was a gradual decrease in the estrogen/progestogen ratio between the first and last day of treatment.

Because large intraindividual variations may render it difficult to find a relationship between the serum levels of contraceptive steroids and intermenstrual bleedings or metabolic serum parameters, the average AUCs of ethinyl estradiol, the progestogens, and the ratios between both were calculated for each individual volunteer on the basis of the 12 days of measurement (Figs. 11 and 12). In both treatment groups there were considerable differences in the average values between the 22 volunteers. As demonstrated by the variances in the ratio between ethinyl estradiol and the progestogens, high or low estrogen levels were not always accompanied by corresponding high or low progestogen concentrations. There was also no correlation between body weight and the AUCs of the contraceptive steroids or the ratios.

When the pharmacokinetics of ethinyl estradiol were investigated during one cycle of treatment with 35 µg of ethinyl estradiol and 2 mg of cyproterone acetate, very large interindividual variations were also found.

One woman had extremely low ethinyl estradiol levels and another woman had very high estrogen concentrations (Fig. 13). There was a significant ($p < 0.01$) increase in estrogen levels between days 1 and 10 (Fig. 14) similar to treatment with ethinyl estradiol/desogestrel and ethinyl estradiol/gestodene.

The results of the single woman who took a combination of 35 µg of ethinyl estradiol and 2 mg of cyproterone acetate and additional gestodene on various days are shown in Fig. 15. There was a pronounced increase in the serum levels of ethinyl estradiol between days 1 and 5 when a steady state appeared to be reached with the preparation alone. The additional intake of gestodene resulted in a further rise in estrogen levels, whereas on those days without gestodene, the ethinyl estradiol concentrations were lower. To investigate the role of rising SHBG levels in the elevation of the serum concentration of gestodene between days 1 and 21 of

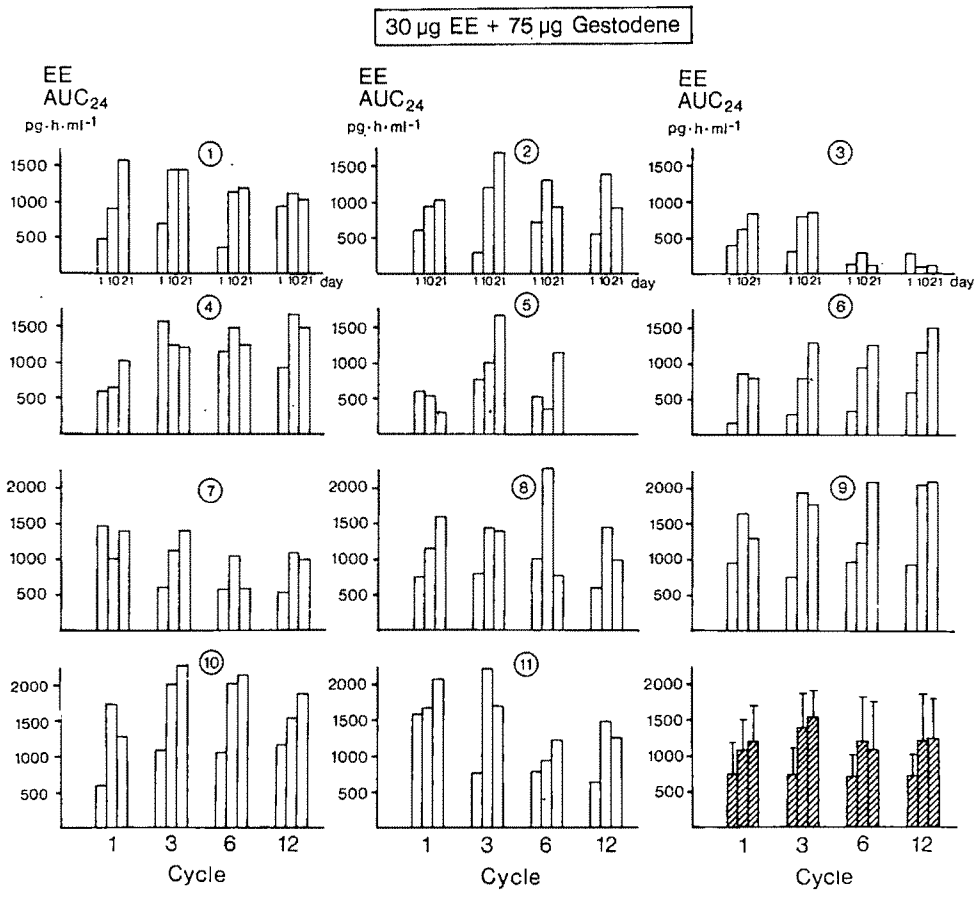


Fig. 8. Individual areas under the time versus serum concentration curves of ethinyl estradiol in 11 volunteers during 12 months of treatment with 30 µg of ethinyl estradiol + 75 µg of gestodene (*hatched columns:* Mean ± SD).

treatment with ethinyl estradiol/gestodene, additional levonorgestrel was taken, which is known to reduce SHBG serum levels because of its androgenic effectiveness. Based on the preceding intake of 35 µg of ethinyl estradiol and 2 mg of cyproterone acetate, SHBG levels were high even after the pill-free interval of 7 days. During treatment with 30 µg of ethinyl estradiol and 75 µg of gestodene and 150 µg of levonorgestrel daily, the initially high SHBG levels decreased by 50% until day 21 (Fig. 16). Nevertheless, gestodene concentrations increased by 35%. When ethinyl estradiol/gestodene was taken alone during the following cycle, the SHBG level increased by 50% between days 1 and 21, whereas the gestodene level rose by more than 300%. During the next pill-free interval of 7 days, she ingested 150 µg of levonorgestrel daily to reduce SHBG level. When she started to again take ethinyl estradiol/gestodene plus 150 µg of levonorgestrel, the gestodene concentrations increased by 100%, whereas SHBG levels rose only slightly until the end of the cycle.

Comment

The present study is the first that investigated in detail the pharmacokinetics of oral contraceptive steroids during a longer time period. The results confirm previous findings of large intraindividual and interindividual variations in the serum levels of ethinyl estradiol and the progestogens. Moreover, there was not only a continuous increase in 3-keto-desogestrel and gestodene levels during multiple intake, which reached a steady state between days 10 and 21, but also a significant rise in ethinyl estradiol concentrations between days 1 and 10. Although maximal progestogen concentrations were reached during the third and sixth cycles,[9, 10] there was no difference in estrogen levels between the first and subsequent treatment cycles.[8] Although the estrogen dose was identical, the observation of significantly higher ethinyl estradiol concentrations during intake of the gestodene-containing pill compared with the combination of ethinyl estradiol with desogestrel indicates an influence of the progestogen

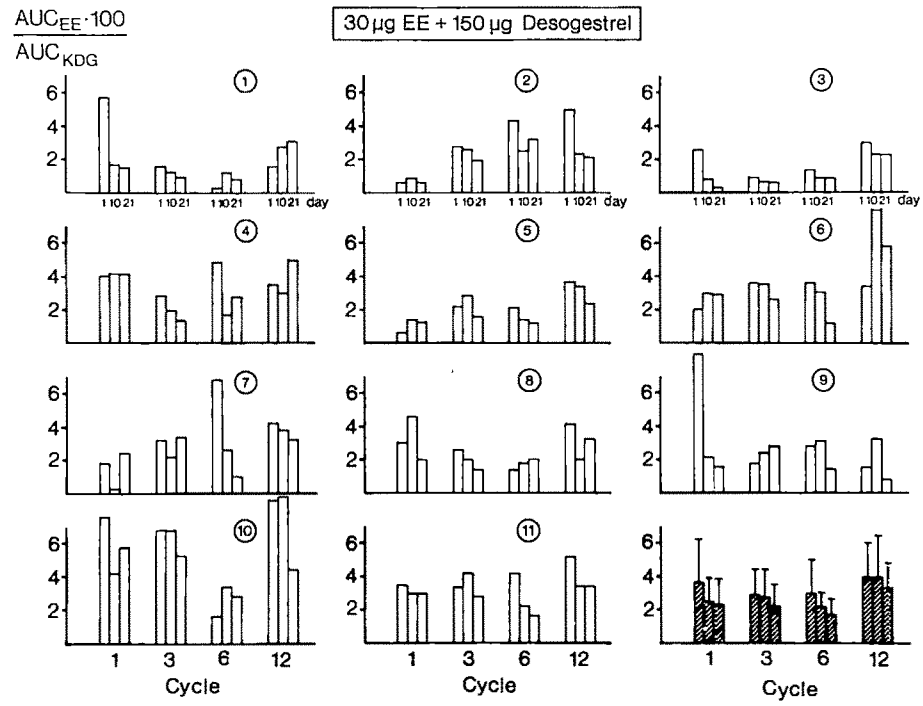


Fig. 9. Individual ratios between the areas under the time versus serum concentration curves of ethinyl estradiol and 3-keto-desogestrel in 11 volunteers during 12 months of treatment with 30 µg of ethinyl estradiol + 150 µg of desogestrel (*hatched columns:* Mean ± SD).

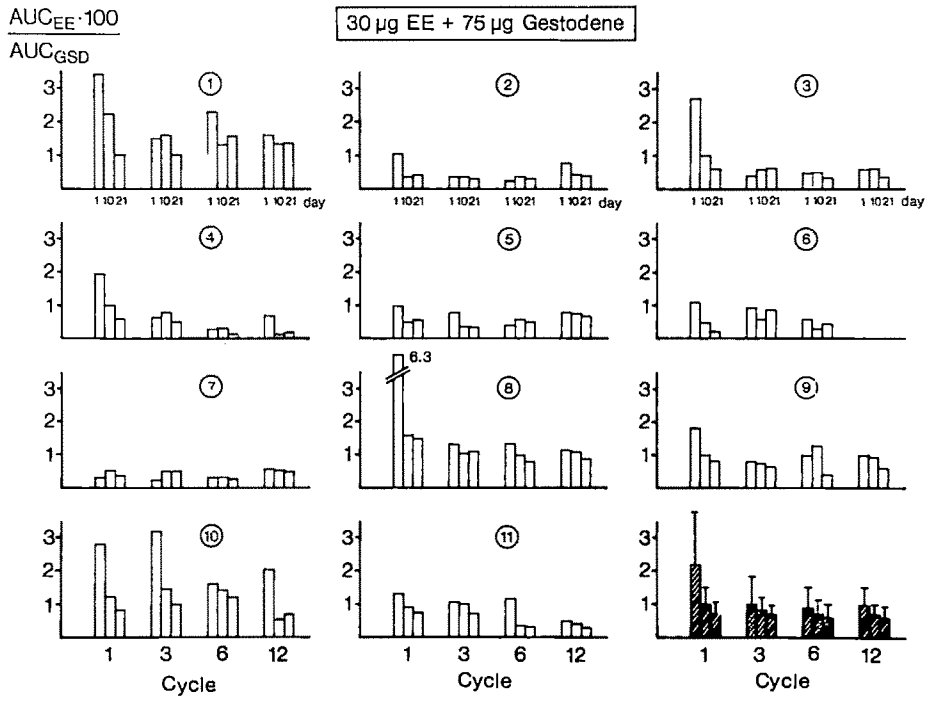


Fig. 10. Individual ratios between the areas under the time versus serum concentration curves of ethinyl estradiol and gestodene in 11 volunteers during 12 months of treatment with 30 µg of ethinyl estradiol + 75 µg of gestodene (*hatched columns:* Mean ± SD).

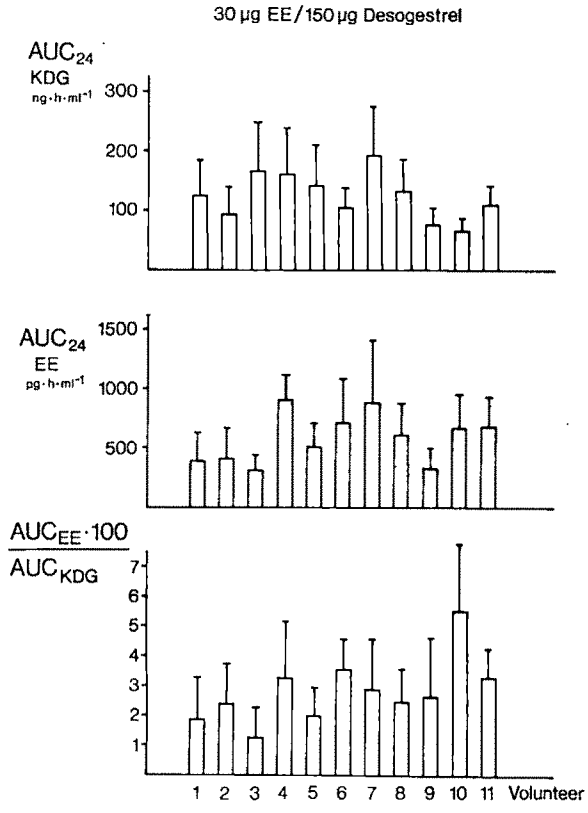


Fig. 11. Average individual areas under the time versus serum concentration curves of 3-keto-desogestrel, ethinyl estradiol, and the ratios between both in 11 volunteers during 12 months of treatment with 30 µg of ethinyl estradiol + 150 µg of desogestrel (mean of 12 measurements ± SD).

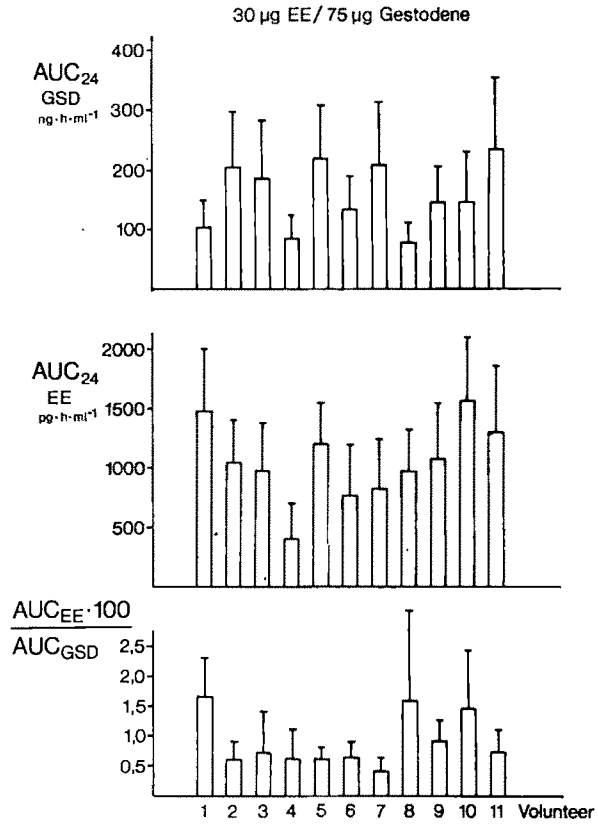


Fig. 12. Average individual areas under the time versus serum concentration curves of gestodene, ethinyl estradiol, and the ratios between both in 11 volunteers during 12 months of treatment with 30 µg of ethinyl estradiol + 75 µg of gestodene (mean of 12 measurements ± SD).

component on the pharmacokinetics of ethinyl estradiol. To date interactions between sex steroids have been known only in regard to the stimulation of serum protein-binding capacity by ethinyl estradiol of nortestosterone derivatives.

Pharmacokinetics of progestogens. The striking difference in the height of the serum concentrations of gestodene and 3-keto-desogestrel was suggested to be exclusively the consequence of the strong binding affinity of gestodene to SHBG, whereas that of 3-keto-desogestrel is much less.[1] Actually, the correlation between SHBG and gestodene was close and that between SHBG and 3-keto-desogestrel was less close.[9, 10] However, correlation does not mean causal relationship. The relative binding affinities to SHBG compared with levonorgestrel were 80% for gestodene and 10% for 3-keto-desogestrel,[11] whereas Bergink et al.[12] found 32% of the binding affinity of levonorgestrel for 3-keto-desogestrel. However, the physiologic significance of binding experiments with dialysis is questionable; it had been demonstrated that, for instance, a low dialyzable percentage of 6% for estradiol does not correlate with the high influx of estradiol into the liver (90%), brain (72%), and uterus (38%) during perfusion experiments.[13] Binding to albumin has nearly no influence on the biologic effectiveness as exemplified with testosterone.[14] The short half-life of testosterone despite its high binding affinity to SHBG, which is threefold that of levonorgestrel, indicates that hepatic metabolism is in all probability the main determinant of the bioavailability of synthetic sex steroids. If the metabolic capacity of hepatic cytochrome P-450–dependent enzymes is the rate-limiting factor, the concentration of free steroid in plasma will be the same as within the hepatocytes,[15] and a slow-down of hepatic inactivation will result in an accumulation of the total steroid level in plasma. A rise in total gestodene concentration implies a corresponding increase in free gestodene levels. The calculation of biologically effective gestodene and 3-keto-desogestrel plasma levels on the basis of the AUCs of the present study and the percentage of non-SHBG-bound gestodene (24%) and 3-keto-desogestrel (68%)[16] revealed that, for example, on day 21 of the third cycle, the non-SHBG-bound amount of gestodene is more

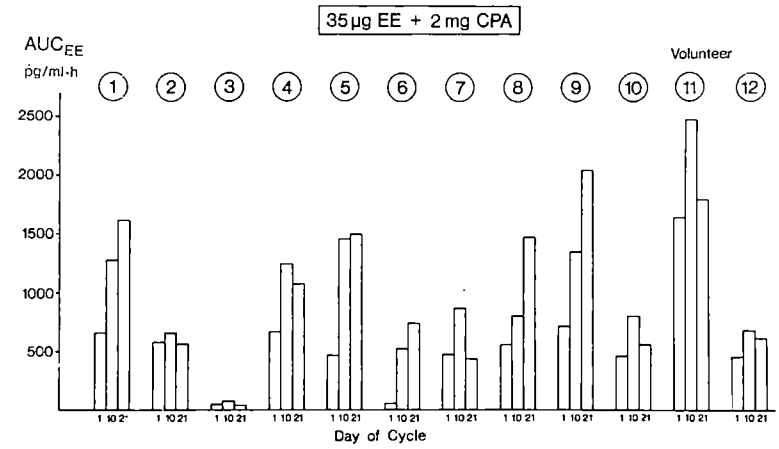


Fig. 13. Individual areas under the time versus serum concentration curves of ethinyl estradiol in 12 volunteers on days 1, 10, and 21 of one cycle of treatment with 35 µg of ethinyl estradiol + 2 mg of cyproterone acetate (CPA).

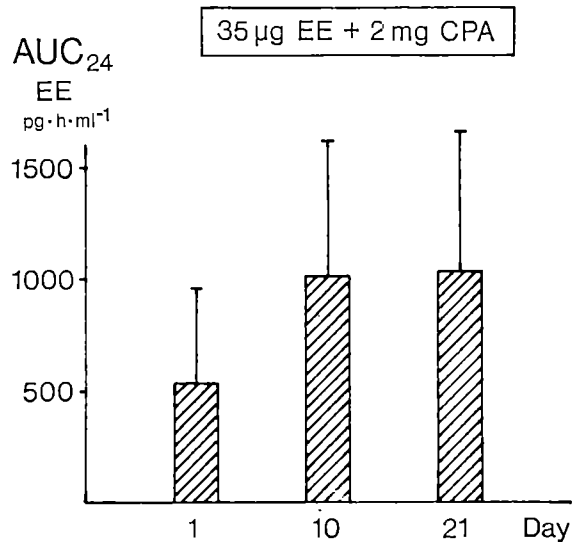


Fig. 14. Areas under the time versus serum concentration curves between 0 and 24 hours of ethinyl estradiol during treatment of 12 women with 35 µg of ethinyl estradiol + 2 mg of cyproterone acetate *(CPA)* for one cycle (mean ± SD).

than twice that of 3-keto-desogestrel (56.6 vs 26.0 ng · hr/ml). With regard to the role of binding of sex steroids to SHBG as a neutralizing factor of biologic effectiveness, recent data throw some doubt on this hypothesis and show that binding to SHBG might be important for an active process of uptake of steroids by target organs.[17, 18]

There are as yet no data on the inactivation rate of the modern progestogens by hepatic enzymes. The significant correlation between the serum concentrations of SHBG and levonorgestrel indicates a pronounced influence of protein binding on metabolic clearance of levonorgestrel.[19-21] On the other hand, two women had no or a negative correlation between SHBG and levonorgestrel levels.[20] In another study there was a continuous increase in levonorgestrel plasma levels throughout a cycle of treatment with 30 µg of ethinyl estradiol and 150 µg of levonorgestrel, although SHBG levels remained unchanged.[22] The authors stated that the rise in levonorgestrel levels cannot be explained by increased binding to SHBG, but it is more likely because of changes in levonorgestrel metabolism. The present results of the time course of SHBG and gestodene during intake of the gestodene-containing pill with or without additional levonorgestrel indicate a strong influence of SHBG binding on gestodene levels, but also the effect of another factor, which is possibly the inhibition of gestodene metabolism.

In a recent study Brody et al.[23] reported on significantly higher norethisterone levels during intake of 50 µg of mestranol and 1 mg of norethisterone compared with 35 µg of ethinyl estradiol and 1 mg of norethisterone, whereas the levels of ethinyl estradiol did not differ. The authors discussed a possible effect of the different estrogens on norethisterone metabolism. It is remarkable that the demethylation of mestranol, which leads to ethinyl estradiol, is catalyzed by cytochrome P-450–dependent enzymes, which also metabolize various steroids and drugs.

Pharmacokinetics of ethinyl estradiol. The significant increase in the levels of ethinyl estradiol, reaching a steady state between days 1 and 10, which can be observed during treatment with different preparations, cannot be the result of a rise in protein binding in serum. Ethinyl estradiol is nearly exclusively bound by albumin, the concentration of which is not influenced by oral contraceptives. It is not probable that an accumulation of ethinyl estradiol sulfate in the blood, which resulted in an elevated enterohepatic recirculation, may be involved, because the sulfates of ethinyl estradiol have been demonstrated not to be slow-release

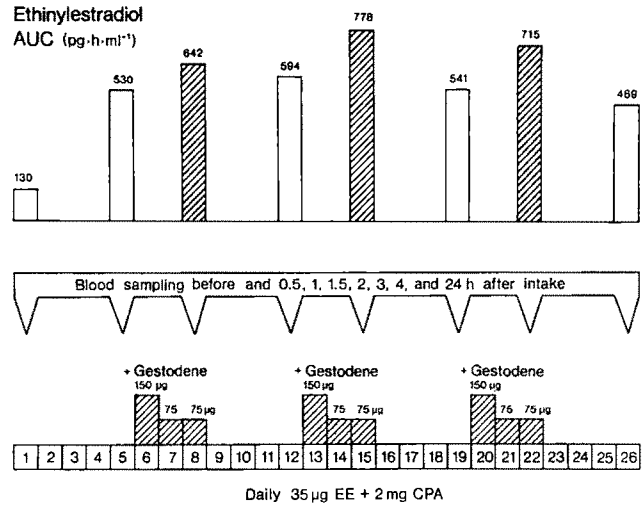


Fig. 15. Areas under the time versus serum concentration curves between 0 and 24 hours of ethinyl estradiol in one woman during treatment with 35 µg of ethinyl estradiol + 2 mg of cyproterone acetate *(CPA)* for 26 days with or without additional gestodene.

reservoirs.[24] The increase in the ethinyl estradiol level and the parallel prolongation of the half-lives of ethinyl estradiol and theophyllin during long-term treatment with an oral contraceptive indicate an inhibition of hepatic metabolism of the steroid and the drug, both of which are inactivated by cytochrome P-450–dependent enzymes.[25] There is a series of drugs, the half-lives of which are increased, and metabolic clearance is decreased during treatment with oral contraceptives.[26] The results of Guengerich[27] indicate that ethinyl estradiol and some progestogens containing an ethinyl group are capable of inhibiting the 2-hydroxylation of ethinyl estradiol. An increase in ethinyl estradiol levels during a cycle of treatment with preparations containing 30 or 50 µg of ethinyl estradiol has also been observed.[28] During multiple intake of daily 30 or 50 µg without progestogens, there was a continuous increase in ethinyl estradiol concentration, which reached a steady state after 7 days with the higher dose and after 12 or more days with 30 µg.[29]

It is improbable that the difference in ethinyl estradiol levels during intake of ethinyl estradiol/desogestrel and ethinyl estradiol/gestodene is because of galenic diversities. The pharmacokinetic parameters of identically composed oral contraceptives of different producers were identical.[23, 30, 31] The higher ethinyl estradiol levels during treatment with the gestodene-containing pill can be explained with an inhibitory influence of gestodene that is circulating in very high concentrations on 2-hydroxylation of ethinyl estradiol. This progestogen has been shown by Guengerich[27] to be an extreme inhibitor of this metabolic reaction in human liver microsomes. It should be kept in mind that during the first liver passage, the serum concentrations of steroids after oral intake are several times higher than those measured peripherally.[32] Therefore during the first passage through the liver, local gestodene concentrations of >100 ng/ml might be reached in the sinusoids, a part of which may penetrate into the hepatocytes and interact with metabolic enzymes. Concerning the physiologic significance of these steroid concentrations, which are much lower than those used in vitro,[27] it is interesting that the endocrine and clinical effects of a new potent aromatase inhibitor, 4-hydroxy-androstenedione, are maintained with relatively low serum levels of 7.8 ± 1.1 ng/ml after intramuscular injection of a microcrystalline suspension.[33] The 4-hydroxy-androstenedione acts as a suicide inhibitor, destroying the cytochrome P-450–dependent aromatase irreversibly.[31]

The supposition can be made that both ethinyl estradiol and nortestosterone derivatives contribute to the inhibition of their metabolism, as well as that of those drugs that are metabolized by cytochrome P-450–dependent enzymes. Regarding the effectiveness of progestogens alone, a decrease in the metabolic clearance rate of aminopyrine by 25% to 35% has been reported in women treated with 0.35 mg of norethisterone daily.[35] The mechanism of the interaction of steroids with cytochrome P-450 enzymes is only partly known. The principal metabolite of ethinyl estradiol, 2-hydroxy-ethinyl estradiol, is further transformed into a highly reactive compound that can irreversibly bind to microsomal protein.[36, 37] The oxidation of the ethinyl group by cytochrome P-450 enzymes leads to very reactive intermediates that can covalently bind to proteins or react with the prosthetic heme group, resulting in an inactivation of the enzyme.[38, 39] During the latter

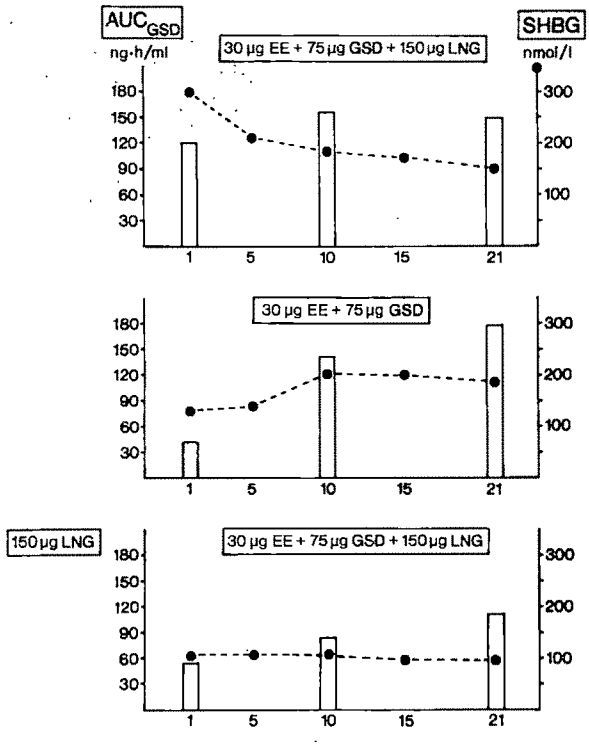


Fig. 16. Areas under the time versus serum concentration curves between 0 and 24 hours of gestodene and serum concentrations of sex hormone–binding globulin during one cycle of treatment with 30 µg of ethinyl estradiol + 75 µg of gestodene + 150 µg of levonorgestrel, during one cycle of treatment with 30 µg of ethinyl estradiol + 75 µg of gestodene and during one cycle of treatment with 30 µg of ethinyl estradiol + 75 µg of gestodene + 150 µg of levonorgestrel after pretreatment with 150 µg levonorgestrel for 7 days in one woman. *GSD,* Gestodene; *LNG,* levonorgestrel.

reaction, which is called mechanism-based inactivation or suicide inhibition of cytochrome P-450–dependent mixed function oxidases, alkylated porphyrins are formed.[40, 41] An increase in urinary excretion of porphyrins has been observed in women during treatment with oral contraceptives.[42, 43] The reactive intermediate after oxidation of the ethinyl group can also react with itself, forming a D-homo-steroid by dilatation of the 5-ring D to a 6-ring. Such metabolites of ethinyl estradiol (D-homo-estradiol) and gestodene have been isolated from urine. Moreover, a high rate of irreversible protein binding of gestodene (= Δ15-d-norgestrel) in the microsomes of rat liver has been previously reported.[44]

Further possible interactions of oral contraceptive steroids with metabolizing enzymes include conjugation of ethinyl estradiol, since estrogen glucuronidation by liver microsomes has been found to be inhibited by ethinyl estradiol.[45] It is known that a major part of ethinyl estradiol, taken orally, is metabolized in the intestinum. Therefore it remains to be elucidated whether an inhibition of intestinal enzymes by contraceptive steroids may play a role. Because it has been demonstrated that progesterone or certain of its metabolites may decrease oxidative drug metabolism,[46] the possible interaction of progesterone derivatives with these enzymes must be the subject of further investigations.

Intraindividual and interindividual variations. The large intraindividual and interindividual variations in the serum concentrations of ethinyl estradiol during treatment with ethinyl estradiol/desogestrel and ethinyl estradiol/gestodene are in agreement with previously reported results of the pharmacokinetic studies on oral contraceptives.[5, 6, 28, 31, 47-50] The reasons for the variability have not as yet been clarified. Multicenter studies demonstrated large variations in the pharmacokinetics of ethinyl estradiol in different countries, but also within the same locality, which indicates that ethnic factors probably play no major role.[47, 51] After adjustment for race, age, weight, height, blood pressure, cigarette and alcohol consumption, or life-time use of oral contraceptives, 72% of the variability in the ethinyl estradiol levels remained unexplainable.[5] Similar to our findings, no influence of body weight could be observed.[28] Contrary to this, a genetic or acquired predisposition seems to have an important influence, since during 12 cycles of treatment with ethinyl estradiol/desogestrel or ethinyl estradiol/gestodene, most of the women showed a relatively stable pattern. However, the large intraindividual variations which were in the same range as the interindividual differences, indicate an important influence of thus far unknown short-lasting factors. In all probability, alterations in the metabolic capacity of the intestinum and liver from day to day appear to be causative, since large variations in the half-life of ethinyl estradiol between 2 and more than 30 hours and in the elimination rate constant between 0.02 and 0.25 have been reported.[23, 50, 51] It has been suggested that the pronounced variability is typical for drugs with a first-pass effect. Ethinyl estradiol is already conjugated to a considerable extent in the intestinal mucosa and the liver.[52] Contrary to this, levonorgestrel, 3-keto-desogestrel, and gestodene are not subject to a first-pass effect. Nevertheless, the average intraindividual and interindividual coefficients of variation of 3-keto-desogestrel and gestodene were in the same range as those of ethinyl estradiol. Similar results have been reported on norethisterone and levonorgestrel.[23, 49, 53, 54] Because of an enhanced enterohepatic circulation, ethinyl estradiol levels may rise secondarily between 5 and 12 hours after food intake.[55] On the other hand, progestogens undergo nearly no enterohepatic circulation because their main metabolic steps are reduction or hydroxylation, which results in a loss of hormonal activity. Moreover, in our study the women fasted overnight. There was no correlation between serum con-

centrations of ethinyl estradiol and those of gestodene or 3-keto-desogestrel. Therefore the coefficient of the ratios between ethinyl estradiol and 3-keto-desogestrel or ethinyl estradiol and gestodene exceeded those of AUCs of the steroids. No correlation between ethinyl estradiol and the respective progestogens has previously been observed during treatment with oral contraceptives.[23, 50]

Environmental factors, such as diet, smoking, and stress, influence oxidative metabolism in humans and animals. Change in posture from lying to standing upright may alter metabolic clearance of androstenedione.[56] The serum level of progesterone is decreased by 34%, and metabolic clearance is increased within 1 hour after a meal, possibly because of enhanced splanchnic blood flow.[57] A high-protein diet leads to an increase in antipyrine oxidation and to an enhancement of 2-hydroxylation of estrogen.[58, 59] Contrary to this, a high-protein diet decreases Δ4-5α-reduction of testosterone.[60] In male chimpanzees, a high-fat diet reduces 2-hydroxylation of estradiol by 50%.[61] An increase in 2-hydroxylation of estradiol was induced by weight loss.[62]

Physical stress diminishes the metabolic clearance rate of estradiol by 80% within a few minutes, which can be explained only partly by a decrease in hepatic blood flow.[63] In male rats the metabolism of drugs and steroids is reduced 48 hours after stress, whereby somatotropic hormone and adrenocorticotropic hormone but not adrenal hormones play an essential role.[64, 65] It is unknown whether the pituitary gland is directly involved in the control of hepatic steroid metabolism in humans.

Pharmacodynamics. During the 12 months of treatment with ethinyl estradiol/desogestrel and ethinyl estradiol/gestodene, no significant difference was found in most of the metabolic serum parameters.[66, 67] This can be explained by the antagonistic effect of the estrogen and progestogen components on lipids, lipoproteins, and most of the hepatic proteins. The effect of higher ethinyl estradiol levels during treatment with ethinyl estradiol/gestodene may be counteracted by a more pronounced action of the high gestodene levels, resulting in a similar net effect such as during intake of ethinyl estradiol/desogestrel. It has been demonstrated that oral contraceptives containing 30 μg ethinyl estradiol and 150 μg of levonorgestrel or 50 μg of ethinyl estradiol and 250 μg of levonorgestrel produced qualitatively and quantitatively similar effects on lipid metabolism, ceruloplasmin, and antithrombin III.[68] The only differences found in our study were significantly higher serum concentrations of corticosteroid-binding globulin and cortisol[8] and a more pronounced increase in triglyceride levels[67] during treatment with the gestodene-containing pill. Corticosteroid-binding globulin is influenced only by the estrogen component and not by progestogens. Moreover, the effects of potent sex steroids are not far from the dose-dependent end-point, and the duration of treatment appears to be more important for the resulting effect on serum parameters than the differences in steroid serum levels. Although steroid levels show large intraindividual variability, the hepatic parameters reflect an integral effect of the steroids over time. This was confirmed in our study by the differences in the corticosteroid-binding globulin and cortisol levels, which became more pronounced during the sixth and twelfth cycles.[8]

In a recent paper the influence of the slope of serum levels of ethinyl estradiol and norethisterone calculated from single determinations before and 1 hour after intake of the last tablet of the third, sixth, and ninth treatment cycles on changes in outlier status for lipids and lipoproteins has been observed.[69] However, the authors did not report on a correlation between steroid levels or slopes and the parameters of lipid metabolism in the total group of volunteers.

In our study no correlation could be found between individual ethinyl estradiol and progestogen levels and the occurrence of intermenstrual bleedings. Neither women with high ethinyl estradiol and low 3-keto-desogestrel or gestodene levels nor women with low estrogen and high progestogen levels showed a consistent bleeding pattern. This was true for the first treatment cycle and also for the whole treatment period when the average of 12 AUCs of ethinyl estradiol and gestodene or 3-keto-desogestrel was compared with the bleeding pattern of each woman. The cause of irregular bleedings has not yet been clarified. It is known that missing one pill during a treatment cycle results in a 13- to 16-fold increase in the risk of intermenstrual bleeding.[70] This means that a pronounced deficiency of circulating steroids on one day may cause bleeding. A similar effect may be produced when relatively high levels (e.g., of the progestogen) during the course of a cycle are superseded by very low levels on one or more days because of the effect of as yet unknown factors on pharmacokinetics. Such an effect cannot be substantiated by single determinations of steroid levels and even not by the investigation of pharmacokinetics on 3 of 21 days of treatment, but reliable data are needed during the whole cycle.

REFERENCES

1. Ahluwalia BS, Curry CL, Crocker CL, Verma PS. Evidence of higher ethynylestradiol blood levels in human hypertensive oral contraceptive users. Fertil Steril 1977; 28:627-30.
2. Kaul L, Curry CL, Ahluwalia BS. Blood levels of ethynylestradiol, caffeine, aldosterone and desoxycorticosterone in hypertensive oral contraceptive users. Contraception 1981;23:643-51.

3. Fahraeus L, Sydsjö A, Wallentin L. Lipoprotein changes during treatment of pelvic endometriosis with medroxyprogesterone acetate. Fertil Steril 1986;45:503-6.
4. Back DJ, Bates M, Burke MW, et al. Plasma Xa inhibitory activities and plasma concentrations of norgestrel and ethinyloestradiol in women on oral contraceptive steroids. Br J Clin Pharmacol 1979;8:505-6.
5. Stadel BV, Sternthal PM, Schlesselman JJ, et al. Variation of ethinylestradiol blood levels among healthy women using oral contraceptives. Fertil Steril 1980;33:257-60.
6. Back DJ, Breckenridge AM, Crawford FE, MacIver M, Orme ML'E, Rowe PH. Interindividual variation and drug interactions with hormonal steroid contraceptives. Drugs 1981;21:46-61.
7. Back DJ, Grimmer SFM, Rogers S, Stevenson PJ, Orme ML'E. Comparative pharmacokinetics of levonorgestrel and ethinyloestradiol following intravenous, oral and vaginal administration. Contraception 1987;36:471-9.
8. Jung-Hoffmann C, Kuhl H. Interaction with the pharmacokinetics of ethinylestradiol and progestogens contained in oral contraceptives. Contraception 1989;40:299-312.
9. Kuhl H, Jung-Hoffmann C, Heidt F. Serum levels of 3-keto-desogestrel and SHBG during 12 cycles of treatment with 30 μg ethinylestradiol and 150 μg desogestrel. Contraception 1988;38:381-90.
10. Kuhl H, Jung-Hoffmann C, Heidt F. Alterations in the serum levels of gestodene and SHBG during 12 cycles of treatment with 30 μg ethinyl-estradiol and 75 μg gestodene. Contraception 1988;38:477-86.
11. Juchem M, Pollow K. Binding of oral contraceptive progestogens to serum proteins and cytoplasmic receptor. Am J Obstet Gynecol 1990;163:2171-83.
12. Bergink EW, Hamburger AD, de Jager E, van der Vies J. Binding of a contraceptive progestogen ORG 2969 and its metabolites to receptor proteins and human sex hormone binding globulin. J Steroid Biochem 1981;14:175-83.
13. Steingold KA, Cefalu W, Pardridge W, Judd HL, Chaudhuri G. Enhanced extraction of estrogens used for replacement therapy. J Clin Endocrinol Metab 1986;62:761-6.
14. Manni A, Pardridge WM, Cefalu W, et al. Bioavailability of albumin-bound testosterone. J Clin Endocrinol Metab 1985;61:705-10.
15. Mendel CM, Kuhn RW, Weisiger RA, et al. Uptake of cortisol by the perfused rat liver: validity of the free hormone hypothesis applied to cortisol. Endocrinology 1989;124:468-76.
16. Kuhnz W, Pfeffer M, Al-Yacoub G. Protein binding of the contraceptive steroids gestodene, 3-keto-desogestrel and ethinylestradiol in human serum. J Steroid Biochem 1990;35:313-8.
17. Sakiyama R, Pardridge WM, Musto NA. Influx of testosterone-binding globulin (TeBG) and TeBG-bound sex steroid hormones into rat testis and prostate. J Clin Endocrinol Metab 1988;67:98-103.
18. Sinnecker G, Hiort O, Mitze M, Donn F, Neumann S. Immunohistochemical detection of a sex hormone binding globulin like antigen in tissue sections of normal human prostate, benign prostatic hypertrophy and normal human endometrium. Steroids 1988;52:335-6.
19. Victor A, Johansson EDB. Effects of d-norgestrel induced decrease in SHBG capacity on the d-norgestrel levels in plasma. Contraception 1977;16:115-23.
20. Victor A, Weiner E, Johansson EDB. Relation between sex hormone binding globulin and d-norgestrel levels in plasma. Acta Endocrinol 1977;86:430-6.
21. Cekan SZ, Jia M, Landgren BM, Diczfalusy E. The interaction between sex hormone binding globulin and levonorgestrel released from vaginal rings in women. Contraception 1985;31:431-9.
22. Song S, Chen J, He M, Fotherby K. Effect of some oral contraceptives on serum concentrations of sex hormone binding globulin and ceruloplasmin. Contraception 1989;39:385-99.
23. Brody SA, Turkes A, Goldzieher JW. Pharmacokinetics of three bioequivalent norethindrone/mestranol-50 μg and three norethindrone/ethinyl estradiol-35 μg OC formulations: are "low-dose" pills really lower? Contraception 1989;40:269-84.
24. Goldzieher JW, Mileikowsky G, Newburger J, Dorantes A, Stavchansky SA. Human pharmacokinetics of ethynyl estradiol 3-sulfate and 17-sulfate. Steroids 1988;51:63-79.
25. Tornatore KM, Kanarkouski R, McCarthy TL, Gardner MJ, Yurchak AM, Jusko WF. Effect of chronic oral contraceptive steroids on the theophylline disposition. Eur J Clin Pharmacol 1982;23:129-34.
26. Teichmann AJ. Influence of oral contraceptives on drug therapy. Am J Obstet Gynecol 1990;163:2208-13.
27. Guengerich FP. Inhibition of oral contraceptive steroid-metabolizing enzymes by steroids and drugs. Am J Obstet Gynecol 1990;163:2159-63.
28. Kaufman JM, Thierry M, Vermeulen A. Plasma levels of ethinylestradiol (EE) during cyclic treatment with combined oral contraceptives. Contraception 1981;24:589-602.
29. Jenkins N, Limpongsanurak S, Fotherby K. Circulating levels of synthetic steroids in women using a "triphasic" formulation: a comparison with different ethinyloestradiol doses. J Obstet Gynaecol 1981;2:37-40.
30. Saperstein S, Edgren RA, Lee GJL, et al. Bioequivalence of two oral contraceptive drugs containing norethindrone and ethinyl estradiol. Contraception 1989;40:581-90.
31. Stanczyk FZ, Lobo RA, Chiang ST, Woutersz TB. Pharmacokinetic comparison of two triphasic oral contraceptive formulations containing levonorgestrel and ethinylestradiol. Contraception 1990;41:39-53.
32. Back DJ, Barkfeldt JO, Breckenridge AM, et al. The enzyme inducing effect of rifampicin in the rhesus monkey and its lack of interaction with oral contraceptive steroids. Contraception 1982;25:307-16.
33. Goss PE, Powles TJ, Dowsett M, et al. Treatment of advanced postmenopausal breast cancer with an aromatase inhibitor, 4-hydroxyandrostenedione: phase II report. Cancer Res 1986;46:4823-6.
34. Miller WR. Fundamental research leading to improved endocrine therapy for breast cancer. J Steroid Biochem 1987;27:477-85.
35. Field B, Lu C, Hepner GW. Inhibition of hepatic drug metabolism by norethindrone. Clin Pharmacol Ther 1979;25:196-8.
36. Bolt HM, Kappus H, Käsbohrer R. Metabolism of 17α-ethinylestradiol by human liver microsomes in vitro: aromatic hydroxylation and irreversible protein binding of metabolites. J Clin Endocrinol Metab 1974;39:1072-80.
37. Purba HS, Maggs JL, Orme ML'E, Back DJ, Park BK. The metabolism of 17α-ethinyloestradiol by human liver microsomes: formation of catechol and chemically reactive metabolites. Br J Clin Pharmacol 1987;23:447-53.
38. Ortiz de Montellano PR. Suicide substrates for drug metabolizing enzymes: mechanisms and biological consequences. In: Gibson GG, ed. Progress in drug metabolism, vol 11. London: Taylor & Francis, 1988:99-148.
39. Guengerich FP. Oxidation of 17α-ethynylestradiol by human liver cytochrome P-450. Mol Pharmacol 1988;33:500-8.
40. White INH, Müller-Eberhard U. Decreased liver cytochrome P-450 in rats caused by norethindrone or ethynyloestradiol. Biochem J 1977;166:57-64.
41. Blakey DC, White INH. Destruction of cytochrome P-450 and formation of green pigments by contraceptive steroids in rat hepatocyte suspensions. Biochem Pharmacol 1986;35:1561-7.
42. Koskelo P, Eisalo A, Toivonen I. Urinary excretion of porphyrin precursors and coproporphyrin in healhy females on oral contraceptives. Br Med J 1966;1:652-4.
43. Burton JL, Loudon NB, Wilson AT. urinary copropor-

phyrin excretion and hepatic function in women taking oral contraceptives. Lancet 1967;2:1326-8.
44. Schuppler J, Günzel P. Liver tumors and steroid hormones in rats and mice. Arch Toxicol 1979;Suppl 2:181-95.
45. Connors S, Vore M. Coregulation of C_3-hydroxyl versus C_{17}-hydroxyl glucuronidation of β-estradiol in pregnancy and after treatment with phenobarbital or ethinylestradiol. J Pharmacol Exp Ther 1988;246:54-9.
46. Soyka LF, Long RJ. In vitro inhibition of drug metabolism by metabolites of progesterone. J Pharmacol Exp Ther 1972;182:320-7.
47. Goldzieher JW, Dozier TS, de la Pena A. Plasma levels and pharmacokinetics of ethynyl estrogens in various populations. I. Ethynylestradiol. Contraception 1980;21:1-16.
48. Brenner PF, Goebelsmann U, Stanczyk FZ, Mishell DR. Serum levels of ethinylestradiol following its ingestion alone or in oral contraceptive formulations. Contraception 1980;22:85-95.
49. Orme ML'E, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1983;8:95-136.
50. Fotherby K. Variability of pharmacokinetic parameters for contraceptive steroids. J Steroid Biochem 1983;19:817-20.
51. Fotherby K, Akpoviroro J, Abdel-Rahman HA, et al. Pharmacokinetics of ethynyloestradiol in women from different populations. Contraception 1981;23:487-96.
52. Back DJ, Breckenridge AM, MacIver M, et al. The gut wall metabolism of ethinyloestradiol and its contribution to the pre-systemic metabolism of ethinyloestradiol in humans. Br J Clin Pharmacol 1982;13:325-30.
53. Fotherby K, Shrimanker K, Abdel-Rahman HA, et al. Rate of metabolism of norethisterone in women from different populations. Contraception 1979;19:39-45.
54. Nair KM, Sivakumar B, Prema K, Rao BSN. Pharmacokinetics of levonorgestrel in Indian women belonging to low socio-economic group. Contraception 1979;20:303-17.
55. Back DJ, Breckenridge AM, Crawfors FE, et al. An investigation of the pharmacokinetics of ethynyl-estradiol in women using radioimmunoassay. Contraception 1979;20:263-73.
56. Flood C, Hunter SA, Lloyd CA, Longcope C. The effects of posture on the metabolism of androstenedione and estrone in males. J Clin Endocrinol Metab 1973;36:1180-8.
57. Nakajima ST, Gibson M. The effect of a meal on circulating steady-state progesterone levels. J Clin Endocrinol Metab 1989;69:917-9.
58. Anderson KE, Conney AH, Kappas A. Nutrition and oxidative drug metabolism in man: relative influence of dietary lipids, carbohydrate, and protein. Clin Pharmacol Ther 1979;26:493-501.
59. Anderson KE, Kappas A, Conney AH, Bradlow HL, Fishman J. The influence of dietary protein and carbohydrate on the principal oxidative biotransformations of estradiol in normal subjects. J Clin Endocrinol Metab 1984;59:103-7.
60. Kappas A, Anderson KE, Conney AH, Pantuck EJ, Fishman J, Bradlow HL. Nutrition-endocrine interactions: induction of reciprocal changes in the Δ^4-5α-reduction of testosterone and the cytochrome P-450-dependent oxidation of estradiol by dietary macronutrients in man. Proc Natl Acad Sci USA 1983;80:7646-9.
61. Musey PI, Collins DC, Bradlow HL, Gould KG, Preedy JRK. Effect of diet on oxidation of 17β-estradiol in vivo. J Clin Endocrinol Metab 1987;65:792-5.
62. Fishman J, Bradlow HL. Effect of malnutrition on the metabolism of sex hormones in man. Clin Pharm Ther 1977;22:721-8.
63. Keizer HA, Kuipers H, Verstappen FTJ, Janssen E. Limitations of concentration measurements for evaluation of endocrine status of exercising women. Can J Appl Spt Sci 1982;7:79-84.
64. Wei E, Wilson JT. Stress-mediated decrease in liver hexobarbital metabolism: the role of corticosterone and somatotropin. J Pharmacol Exp Ther 1971;177:227-33.
65. Colby HD, Gaskin JH, Kitay JI. Effects of anterior pituitary hormones on hepatic corticosterone metabolism in rats. Steroids 1974;24:679-86.
66. Jung-Hoffmann C, Heidt F, Kuhl H. Effect of two oral contraceptives containing 30 μg ethinylestradiol and 75 μg gestodene or 150 μg desogestrel upon various hormonal parameters. Contraception 1988;38:593-603.
67. März W, Jung-Hoffmann C, Heidt F, Gross W, Kuhl H. Changes in lipid metabolism during 12 months of treatment with two oral contraceptives containing 30 μg ethinylestradiol and 75 μg gestodene or 150 μg desogestrel. Contraception 1990;41:245-58.
68. Nash AL, Cornish EJ, Hain R. Metabolic effects of oral contraceptives containing 30 micrograms and 50 micrograms of oestrogen. Med J Aust 1979;2:277-81.
69. Burkman RT, Zacur HA, Kimball AW, Kwiterovich P, Bell WR. Oral contraceptives and lipids and lipoproteins: Part II—relationship to plasma steroid levels and outlier status. Contraception 1989;40:675-89.
70. Talwar PP, Dingfelder JR, Ravenholt RT. Endometrial control: a comparative study of three oral contraceptives. Int J Gynaecol Obstet 1976;14:385-9.

Prodrugs: Advantage or disadvantage?

Jürgen Hammerstein, MD
Berlin, West Germany

Our knowledge of the peculiarities of prohormones is rather limited, both pharmacologically and clinically. Generalizations cannot be made except that the lapse of time until peak blood values of the active drug have been reached are always greater after intake of the prodrug than after intake of the drug. This finding is presumably of no clinical importance. If pharmacokinetic differences are limited to the phase of distribution, bioequivalence may be assumed. If, on the other hand, the area under the curve during the elimination phase is smaller for the prodrug than for the drug, the potency of the former should be decreased. A shift in the spectrum of endocrine actions as a result of the biotransformation of the prodrug into the active drug is rather the exception than the rule, and so is a change in side effects. If there are major differences in this respect, metabolic pathways in addition to those leading to the respective active drug must also be taken into consideration. (Am J Obstet Gynecol 1990;163:2198-203.)

Key words: Contraceptive prodrugs, estrogens, progestogens

Hormonal prodrugs (prohormones) exert their specific effects only after biotransformation. The development of the first hormonal drugs of this sort dates back to the 1950s, when the concept of prodrugs was still unknown. Thus, lynestrenol (LYN) appeared at that time to be a potent progestogen by itself, despite lacking an oxygen in 3-position—a configuration that had already been recognized at that time to be a prerequisite of the endocrine activity of a given steroid hormone. The prodrug nature of LYN was disclosed only years later when this steroid was found to be quickly biotransformed into norethisterone before action. The same was found to be true for other members of the estrane category, that is, for norethisterone acetate (NETA) and ethynodiol diacetate (EDD). Among the gonanes, desogestrel and norgestimate are prohormones, and even cyproterone acetate (CPA), a progestogen of the pregnane category, may be considered a borderline prohormone.

Mestranol is another example of an old pharmaceutical compound, the prodrug nature of which was not detected before the receptors came into play years later. By definition, pure prodrugs like mestranol, LYN, EDD, and desogestrel do not interact with the receptor at all. There are also incomplete prodrugs such as NETA and norgestimate, with receptor binding affinities being more or less limited (Table I).

Whether the use of prodrugs instead of drugs is an advantage will be discussed under pharmacokinetic, quantitative, endocrine, and metabolic aspects. This issue can be properly investigated only if the active drug is available for comparison, as for example, norethisterone (NET) or ethinyl estradiol (EF_2). Altogether, relevant data are sparse, and only occasionally are true comparisons done.

Table I. Relative binding affinities of various progestational drugs, prodrugs, and intermediates

	Receptor	
	Progesterone	*Androgen*
	(Promegestron R 5020 = 100)	*(Metribolon R 1881 = 100)*
Progesterone	50	6,6
NET	75	15
NETA	20	5
LYN	1	0,7
EDD	1	0
Norethynodrel	6	0
Desogestrel	3	0
3-α-OH-desogestrel	5	0
3-β-OH-desogestrel	13	3
3-Ketodesogestrel	150	16
Norgestrimate	15	—
Norgestrel-17-β-acetate	135	—
Norgestrel-3-oxim	10	—
Levonorgestrel	150	30

Data from Kuhl H. Wien Med Wschr 1987;137:433-40.

Concerning the estranes, to begin with the oldest category of oral progestational compounds, the pharmacokinetic data are scattering at a wide range (Table II). As the only reproducible pharmacokinetic peculiarity, time interval to peak (T_{max}) of NET after oral ingestion of 1 mg of NETA, EDD, or LYN occurs about an hour later than that after oral intake of NET itself. Thus, biotransformation of these prodrugs into NET obviously takes at least 1 hour. Furthermore, half life

From the Division for Gynecologic Endocrinology, Sterility and Family Planning, Obstetrics and Gynecology Department, Klinikum Steglitz, Freie Universität Berlin.
Reprints are not available.
6/0/24860

Table II. Pharmacokinetics of norethisterone after oral administration of various estranes (1 mg/dl)

Drug given	*NET*			*NETA**	*EDD*	*LYN*
AUC_{0-24} (ng*h/ml)	84 ± 6	34.9 ± 12.4	108	172.2 ± 52.0	38.6-43.2	64.1 ± 46.5†
C_{max} (ng/ml)	15.7 ± 1.26	3.2-6.1	14.5	18.3 ± 4.8	6.5-7.4	14.6 ± 9.9
C_{24} (ng/ml)	0.72 ± 0.079	<1.0	0.91 ± 0.46	—	0.3 ± 0.3	—
T_{max} (h)	1.17	1-2	1.22	2	2.21-2.4	2.5 ± 1.7
Source (reference number)	23	29	30	31	32	—

AUC_{0-24}, Area under curve, 0 to 24 hours; *C_{max}*, maximal concentration; *C_{24}*, concentration after 24 hours; *T_{max}*, time to peak interval.

*Fifteenth day of treatment.

†AUC_{0-12}

Table III. Progestational potencies relative to norethisterone (= 1) of various progestogens in human subjects, depending on the parameters tested

Progestogen	*Delay of menses*			*Transformation* §	*Glycogen deposition* ‖	*Ovulation inhibition* ¶
	*	†	‡			
Norethisterone	1	1	1	1	1	1
Norethisterone acetate	0.5	1.6		2	1.3	1
Lynestrenol			1.5	2-3	0.75	
Ethyndioldiacetate	0.5	5		10	1.5	
Levonorgestrel	5		30	17-25	2.3	10
Medroxyprogesterone acetate		1		3		
Chlormadinone acetate		1		5		0.35

*Data from Swyer GIM. Contraception 1982;26:23-7.

†Data from Greenblatt RB. Med Sci 1967;18:37-49.

‡Data from other sources.

§Data from various sources.

‖Data from Ferin J. In: Taust M, ed. International encyclopedia of pharmacology and therapeutics, vol II. Oxford: Pergamon Press, 1972.

¶Data from Neumann F, Elgar W, Nishino Y, Steinbeck H. Arzneimittel-Forschung 1977;27:296-318.

between 24 and 27 hours after oral intake of 5 mg LYN turned out to be definitely longer than after 5 mg NET in the five patients tested.[1]

The areas under the plasma curve (AUC) of NET after ingestion of 0.3 mg NET and 0.5 mg LYN were found to be almost equal. Compatible AUCs were also seen after oral intake of 0.7 mg NET and 1.0 mg LYN. At a daily dose of 5 mg of either drug, the AUC between 0 and 24 hours was about 20% higher after NET than after LYN.[1,2] Thus, biotransformation of LYN into NET does not appear to be complete.

As long as 120 hours after administration of carbon 14–labeled LYN, plasma radioactivity is still rather high, which again indicates that a minor portion of LYN may escape metabolization into NET. Furthermore, because a shift occurs of T_{max} to longer time intervals with increasing oral doses of LYN, it is tempting to assume that the capacity of the liver to transform LYN into NET is limited. In other words, high-dose LYN medications might constitute a liver burden.[3]

After three decades of widespread use of LYN for contraceptive as well as gynecologic purposes, evidence is still lacking as to whether any clinical impact exists in the pharmacokinetic differences between LYN and NET. This is in particular true for the somewhat longer biologic half-life and the slightly lower AUC after oral intake of LYN than after that of NET. Thus, the use of LYN instead of NET implicates some uncertainties without clear-cut advantages or disadvantages.

No biologic tests are available to measure with any degree of reliability the progestational potency of drugs in women. As shown in Table III, results in this field are rather controversial. Thus, it cannot be stated which is more potent, the prodrugs of NET or the drug itself, to say nothing about ranking. The progestational strength of EDD, for example varies relatively to NET by factor 20, despite the fact that the AUCs have been reported to be rather similar.[4]

Norethynodrel is another member of the estrane family that needs attention. The unchanged drug can be detected in the plasma for only 30 minutes.[5] Conversion into NET occurs among other sites in the endometrium and myometrium, with a degree of activity being higher during the proliferative than during the secretory phase.[6,7] It is generally believed that this drug does not exert its progestational activity before transformation into NET. In contrast to NET and the other estranes, however, this prohormone is free of androgenic activity. Also, it possesses a much stronger estrogenic potency than the other estranes, as was demon-

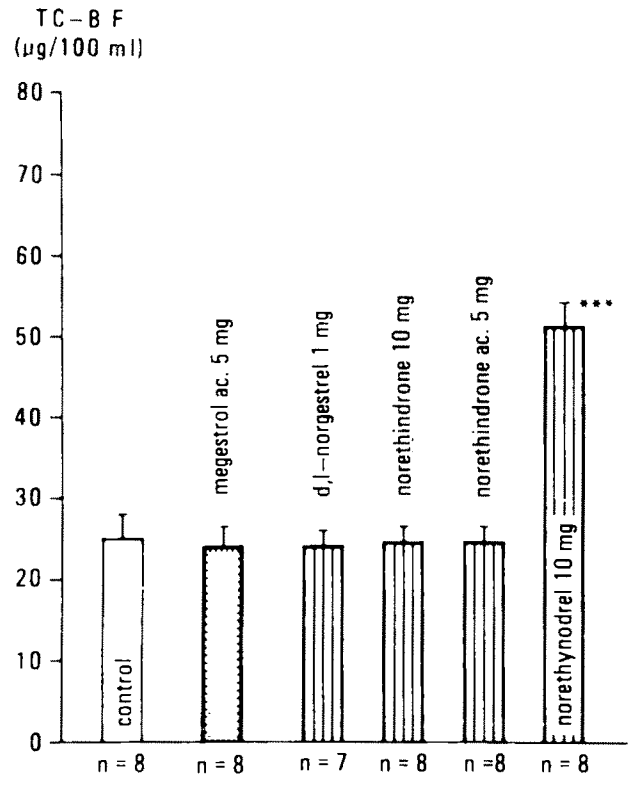


Fig. 1. Cortisol-binding capacity of transcortin *(TC-BF)* as influenced by various contraceptive progestogens administered successively for 14 days each in eight (seven) women with total gonadal insufficiency. (From Schwartz U, Hammerstein J. Acta Endocrinol 1974;76:159-71. By permission.)

strated, among other things, on the basis of the mouse uterine test.[8] When transcortin binding capacity was used as an indicator of estrogenicity, the same was also shown in women: out of five progestogens, norgestimate was the only one to increase this plasma parameter to an extent similar to that of EE_2 (Fig. 1). Thus, norgestimate must be biotransformed not only into NET but also along other routes, unless one distrusts the producer's affirmation that this first orally applicable progestogen was free of estrogenic contaminations. Ethynodiol, the metabolic intermediate between norgestimate and NET, should be without relevance in this respect because it is also an intermediate of LYN.

Desogestrel must be biotransformed through 3-α-OH-desogestrel and 3-β-OH-desogestrel into 3-ketodesogestrel to act as a progestogen at the target cell level (Table I). This transformation is a matter of approximately half an hour; thereafter, plasma levels of 3-ketodesogestrel are almost identical irrespective of whether desogestrel or 3-ketodesogestrel has been administered at the same dosage level (Fig. 2). Nothing has become known as to whether 3-ketodesogestrel in clinical terms is as good a contraceptive progestogen as desogestrel and whether it is tolerated equally well.

Informations are limited concerning norgestimate. No doubt this progestogen of the gonane category is biotransformed into levonorgestrel, to what degree, however, is a matter of debate. In particular, it is still unknown whether norgestimate itself and/or its transformation products norgestrel-3-oxim and norgestrel-17-β-acetate are also endocrinologically active because they bind also to the receptors more or less tightly (Table I).

The field of progestational agents should not be left without—at least briefly—mentioning cyproterone acetate (CPA). CPA may be classified as a borderline prodrug because the major part is biotransformed into the 15-β-OH-analogue (15-OH-CPA), which still—like the parent substance—exerts progestational as well as antiandrogenic actions (Fig. 3). To judge from animal experiments, the progestational and antiandrogenic potencies are balanced much better with 15-OH-CPA than with the parent compound, in which the former property outweighs the latter considerably.[9] 15-OH-CPA is thus an example of a "drug" that appears to be advantageous over the "prodrug." This track has not been followed further by the producer, unfortunately.

The vivid debate in the past as to whether mestranol and EE_2 are equal both in quantitative and qualitative respect has faded away in recent years without coming to a definite conclusion. Because various drug regulatory authorities felt the two estrogenic drugs were bioequivalent and the majority of pharmaceutical companies believed the opposite to be true, mestranol has not been introduced into low-dose oral contraception. This issue, therefore, is only of academic interest today.

To judge from EE_2 plasma levels after oral ingestion of the two estrogenic drugs, the bioavailability of EE_2 has unanimously been found to be higher than that of mestranol. Also, T_{max} was distinctly shorter after EE_2 than after mestranol administration.

According to unpublished data of this meeting, the area under the EE_2 curve after mestranol ingestion is only two thirds that after EE_2 intake, and the same appears to be true for the peak values.[10]

This is in accord with experimental data of Bolt and Bolt,[11] who determined the in vivo demethylation rate of mestranol to EE_2 to be 53.7%, with the residual mestranol undergoing hydroxylation and conjugation before elimination. As another difference, the metabolic clearance rate of mestranol was found to be 1741 ± 393 L/24 h; for EE_2 it was 1345 ± 221 L/24 h.[12] The clearance rates of the metabolites of radioactive mestranol, on the other hand, were shown to be slower than those of the metabolites of radioactive EE_2.[13]

In keeping with these findings are data from their own laboratory on cortisol binding capacity of transcortin as influenced by these two estrogens after oral application. When using this parameter, mestranol had only two thirds the potency of EE_2 (Fig. 4). Histometric

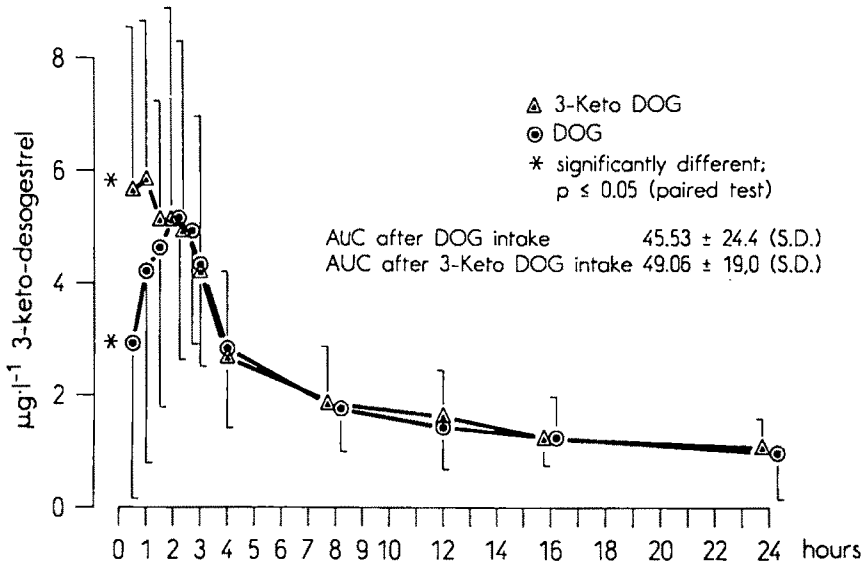


Fig. 2. Pharmacokinetics of 3-ketodesogestrel after single administration of 150 gm desogestrel or 150 gm 3-ketodesogestrel together with 30 gm EE_2 each. (Modified from Hasenack HG, Bosch AMG, Käär K. Contraception 1986;33:591-6, by permission of the copyright holder, Butterworth-Heinemann.)

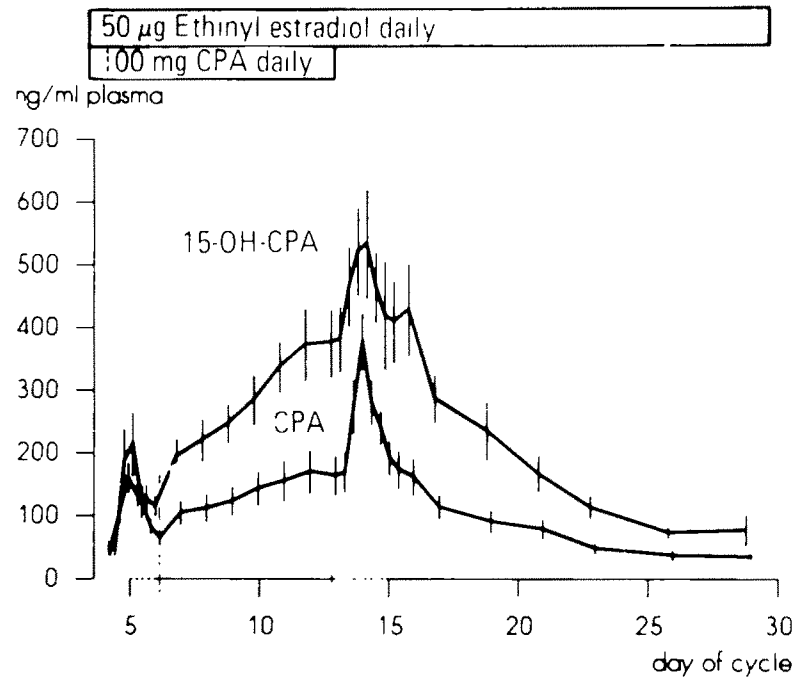


Fig. 3. Pharmacokinetics of CPA and its 15-β-OH-metabolite as measured by high-performance liquid chromatography in the course of the reverse sequential therapy in seven androgenized patients. Data are expressed as mean ± SEM, diurnal course on day 5 and 15 of cycle. (From Hammerstein J. Antiandrogens: clinical aspects. In: Orfanos C, Happle R, eds. Hair and hair diseases. Berlin, West Germany: Springer, 1990:827-86, by permission.)

as well as histologic endometrial findings are also well in accordance with the above.[14, 15]

No differences in potency between mestranol and EE_2, on the other hand, were found by others, based either on vaginal cytology,[16] on inhibition of gonadotropin secretion,[17, 18] on suppression of ovulation, or on endometrial findings.[19, 20] The lack of differences in potency between the two estrogens in these investigations might have been caused by estrogen dosage being too high for the establishment of an appropriate dose-response relationship under in vivo conditions.

In clinical terms, a clear-cut distinction between the

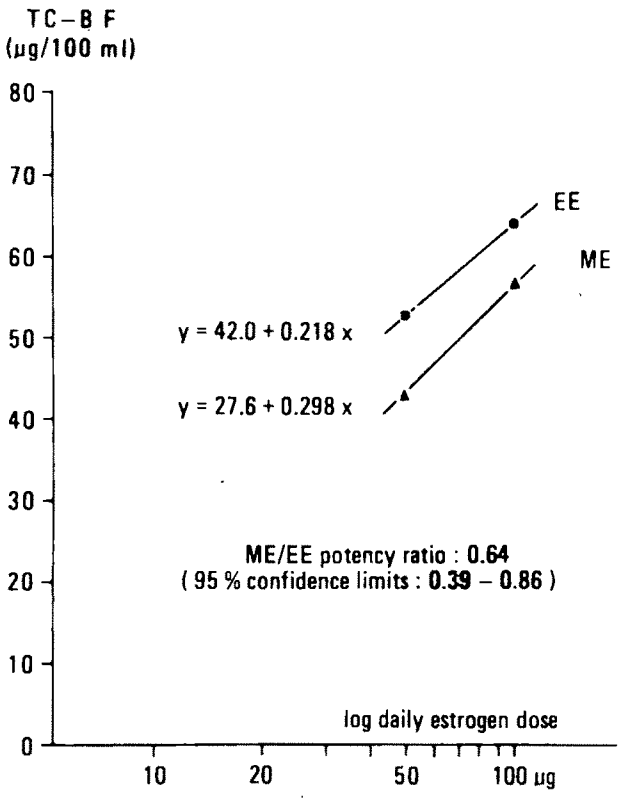


Fig. 4. Cortisol-binding capacity of transcortin as influenced by mestranol *(ME)* and ethinyl estradiol *(EE)*, successive measurements after 14 days of administration of each of the two estrogens at two dosage levels in four women with total gonadal insufficiency. (From Schwartz U, Hammerstein J. Acta Endocrinol 1974;76:159-71. By permission.)

two oral contraceptive estrogens cannot be made. According to the well-known British-Scandinavian study of Inmann et al.,[21] the incidence of thromboembolism appears to be positively correlated with the dose of the estrogen component of the pill, with no difference between EE_2 and mestranol being discernible. Contrary to earlier suggestions, the influence of the two estrogenic drugs on carbohydrate metabolism compares well, and so does their influence on triglycerides.[22, 23]

REFERENCES

1. Odlind V, Weiner E, Victor A, et al. Plasma levels of norethindrone after single dose administration of norethindrone and lynestrenol. Clin Endocrinol (OXF) 1979; 10:29-38.
2. Kuhl H, Bremser HJ, Taubert HD. Serum levels and pharmacokinetics of norethisterone after ingestion of lynestrenol: its relation to dose and stage of the menstrual cycle. Contraception 1982;26:303-15.
3. Hümpel M, Wendt H, Dogs G, et al. Intraindividual comparison of pharmacokinetic parameters of D-norgestrel, lynestrenol and cyproterone acetate in 6 women. Contraception 1977;16:199-215.
4. Walls C, Vose CW, Horth CE, et al. Radioimmunoassay of plasma norethisterone after ethynodiol diacetate. J Steroid Biochem 1977;8:167-71.
5. Cook CE, Twine ME, Tallent CR, et al. Norethynodrel metabolites in human plasma and urine. J Pharmacol Exp Ther 1972;183:197-205.
6. Martin AP, Halterman DR, Vorbeck ML, et al. Metabolism of norethynodrel, a 19-NOR progestin: subcellular localization of enzyme activity. Steroids 1970;16:487-93.
7. Orme ML, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1983;8:95-136.
8. Weems Chihal HJ, Peppler RD, Dickey RP. Estrogen potency of oral contraceptive pills. Am J Obstet Gynecol 1975;121:75-83.
9. Schleusner A, Nishino Y, Albring M, et al. Endocrine profile of a cyproterone metabolite and its potential clinical use. Acta Endocrinol (Copenh) 1980;94(suppl 234): 106.
10. Goldzieher JW, Brody S. Pharmacokinetics of ethinyl estradiol and mestranol. Am J Obstet Gynecol 1990;163: 2114-9.
11. Bolt HM, Bolt WN. Pharmacokinetics of mestranol in man in relation to its oestrogenic activity. Eur J Clin Pharmacol 1974;7:295-305.
12. Bird CE, Clark AF. Metabolic clearance rates and metabolism of mestranol and ethynyl estradiol in normal young women. J Clin Endocrinol Metab 1975;36:296-302.
13. Mills TM, Lin TJ, Braselton WE, et al. Metabolism of oral contraceptive drugs. The formation and disappearance metabolites of norethindrone and mestranol after intravenous and oral administration. Am J Obstet Gynecol 1976;126:987-92.
14. Delforge JP, Ferin J. A histometric study of two estrogens: ethinyl-estradiol and its 3-methyl-ether-derivative (mestranol); their comparative effect upon the growth of the human endometrium. Contraception 1970;1:57-72.
15. Henzl MR, Moyer DL, Townsend D, et al. Quantitation of the estrogenic effects of mestranol on human endometrium and vaginal mucosa. Am J Obstet Gynecol 1973;115:401.
16. Teter J, Stubnicki R. A comparative study of the estrogenic potential of two synthetic estrogens (mestranol and ethinylestradiol). Acta Cytologica 1971;15:167-70.
17. Franchimont P, Legros VV, Meurice J. Effect of several estrogens on serum gonadotropin levels in postmenopausal women. Hormone Metab Res 1972;4:288.
18. Goldzieher JW, Maqueo M, Chenault CB, et al. Comparative studies of the ethynyl estrogens used in oral contraceptives. I. Endometrial response. Am J Obstet Gynecol 1975;122:615-8.
19. Goldzieher JW, Pena A, Chenault CB, et al. Comparative studies of the ethynyl estrogens used in oral contraceptives. II. Antiovulatory potency. Am J Obstet Gynecol 1975;122:619-24.
20. Goldzieher JW, de la Pena A, Chenault CB, et al. Comparative studies of the ethynyl estrogens used in oral contraceptives. III. Effect on plasma gonadotropins. Am J Obstet Gynecol 1975;122:625-36.
21. Inmann WHW, Vessey MP, Westerholm B, et al. Thromboembolic disease and the steroidal content of oral contraceptives. A report to the committee on safety of drugs. Br Med J 1970;2:203-9.
22. Spellacy WM, Buhi WC, Birk SA. The effect of estrogens on carbohydrate metabolism, glucose, insulin and growth hormones. Studies in one-hundred-and seventy-one women ingesting Premarin, mestranol and ethinylestradiol for 6 months. Am J Obstet Gynecol 1972; 114:378-92.
23. Stokes T, Wynn V. Serum lipids in women on oral contraceptives. Lancet 1971;2:677-81.
24. Kuhl H. Progestagene zur empfängnisverhütung. Wien Med Wschr 1987;137:433-40.
25. Schwartz U, Hammerstein J. The oestrogenic potency of various contraceptive steroids as determined by their effects on transcortin-binding capacity. Acta Endocrinol 1974;76:159-71.

26. Hasenack HG, Bosch AMG, Käär K. Serum levels of 3-ketodesogestrel after oral administration of desogestrel and 3-ketodesogestrel. Contraception 1986;33:591-6.
27. Hammerstein J. Antiandrogens: clinical aspects. In: Orfanos C, Happle R, eds. Hair and hair diseases. Berlin, West Germany: Springer, 1990:827-86.
28. Stanczyk FZ, Mroszczak EJ, Ling T, et al. Plasma levels and pharmacokinetics of norethindrone and ethinylestradiol administered in solution and as tablets to women. Contraception 1983;28:241-51.
29. Kiriwat O, Fotherby K. Pharmacokinetics of oral contraceptive steroids after morning or evening administration. Contraception 1983;27:153-60.
30. Saperstein S, Edgren RA, Ellis DJ, et al. Bioequivalence of norethindrone and ethinyl estradiol for two different weight tablets with the same hormonal content. Contraception 1986;33:547-57.
31. Sakar NN, Laumas V, Agarwal N, et al. Norethindrone in serum after use of an oral contraceptive containing norethindrone acetate. Acta Obstet Gynecol Scand 1983; 62:71-6.
32. Vose CW, Butler JK, Williams BM, et al. Bioavailability and pharmacokinetics of norethisterone in women after oral doses of ethynodiol diacetate. Contraception 1979; 19:119-27.

Gastrointestinal disease and oral contraception

Jürgen P. Hanker, MD
Münster, West Germany

Oral contraceptive steroids play a major role in modern family planning. With the present tendency to decrease the doses of both estrogens and progestogens, any factor that reduces the bioavailability of the lower-dose preparations may have an impact on contraceptive protection. Although ethinyl estradiol, the most commonly used oral estrogen, is liable to an enterohepatic circulation as unchanged drug, the commonly used progestogens are not. At present, no convincing evidence exists in the human subject that disruption of the enterohepatic circulation by antibiotics or antacids does reduce contraceptive efficacy of the pill. Oral contraceptive steroids are mainly absorbed from the small bowel, and contraceptive efficacy depends on its absorptive capacity. Enhanced passage of gastrointestinal contents or impaired absorption may thus contribute to contraceptive failures in patients who have chronic inflammatory disease, diarrhea, ileostomy, or jejunoileal bypass. (Am J Obstet Gynecol 1990;163:2204-7.)

Key words: Oral contraceptive steroids, gastrointestinal disease, contraceptive failure

Oral contraceptive steroids have now been in widespread use for more than two decades. It is estimated that worldwide these drugs are regularly taken by more than 60 million women.[1] Over the past few years, a large body of information has appeared dealing with the clinical pharmacology of these substances in young and healthy women. In contrast, the knowledge about the pharmacokinetics of oral contraceptive steroids in disease states is only limited. To reduce toxicity of oral contraceptive preparations, both estrogenic and progestogenic components have gradually been reduced. Most contraceptive formulations now contain <50 μg estrogen and 1 mg progestogen or less. Any factor that reduces the bioavailability of these lower-dose preparations may thus become very relevant in terms of contraceptive protection. Dietary factors, gastrointestinal disturbances or diseases, or drugs that interact with contraceptive steroids and thereby reduce their efficacy may constitute some of these factors. Because oral contraceptive users undergo prolonged periods of steroid ingestion, they most likely experience dietary or gastrointestinal problems or coadministration of an additional drug at some time during oral contraception.

From the Zentrum für Frauenheilkunde of the Westfälische Wilhelms-Universität.
Reprint requests: Jürgen P. Hanker, MD, Zentrum für Frauenheilkunde, Albert-Schweitzer-Strasse 33, D-4400 Münster, West Germany.
6/0/24556

Enterohepatic circulation of oral contraceptive steroids

Oral contraceptive steroids are of two types: estrogens and progestogens. Of the estrogens, ethinyl estradiol is the most important. Less commonly used is mestranol, the methylated derivative of ethinyl estradiol.

Ethinyl estradiol is rapidly absorbed in humans, and peak concentrations of the drug are reached by 120 minutes after variable dosing.[2-9] After a single oral dose of 50 μg, peak concentrations of ethinyl estradiol range from 100 to 200 pg/ml.[10] During long-term treatment with 30 to 50 μg ethinyl estradiol daily, a plasma concentration range of 6 to 190 pg/ml is found.[11]

Ethinyl estradiol undergoes considerable first-pass metabolism,[12] of which the chief reaction is sulfate conjugation in jejunal mucosa.[13] Gut wall conjugation accounts for about 65% of the first-pass elimination of the drug.[14] Thus bioavailability of an oral preparation relative to an intravenous one is 40% on average; the interindividual variation, however, is great (20% to 65%).[2] Preliminary data suggest that gut wall metabolism of ethinyl estradiol is reduced in patients with celiac disease, thereby increasing the bioavailability of the steroid.[13] However, the clinical relevance of this observation has yet to be confirmed.

Because of the 17α-ethinyl group, ethinyl estradiol is relatively resistant to metabolism by 16α-hydroxylation, which in the case of estradiol itself leads

to the formation of 16α-hydroxyestrone and estriol. Thus the substance is metabolized chiefly by aromatic hydroxylation, resulting in the most important ring, A metabolite 2-hydroxy-ethinyl estradiol.[15, 16] Additionally, ethinyl estradiol may be hydroxylated in the C_4, C_6, and C_{16} positions,[17, 18] and 6α- and 16β-hydroxy-ethinyl estradiol are formed. These metabolites are conjugated chiefly with glucuronide.[19]

Ethinyl estradiol is excreted in bile and urine principally as glucuronide or sulfate conjugates.[20] Conjugation takes place largely on the 3 position. After excretion in the bile,[21] these conjugates reach the gut lumen and are hydrolyzed by enzymes present in gut bacteria. Thus the original compound is liberated and may be reabsorbed.[22] This mechanism forms the basis for an enterohepatic circulation of ethinyl estradiol as an unchanged drug. At present it is not clear to what extent the enterohepatic recirculation contributes to the overall plasma concentration profile of ethinyl estradiol in the human female.[10] If one assumes that it does, any disruption of this mechanism should increase the risk of contraceptive failure.

Oral treatment with antibiotics would be expected to interfere with enzymatic hydrolysis and hence reduce or abolish steroid reabsorption from the gut. It is the unconjugated steroid only that can be reabsorbed across the intestinal mucosa. Reports exist in the literature that link oral antibiotic therapy to contraceptive failures in oral contraceptive users.[10, 23-26] However, clinical studies with ampicillin have failed to demonstrate any significant interaction between the antibiotic and oral contraceptive steroids.[27-30]

Prolonged ampicillin therapy will rapidly lead to resistant organisms in the gut. Resistance to ampicillin may develop within 5 to 6 days of therapy.[31] Any significant effect on ethinyl estradiol metabolism may, therefore, have been missed if monitoring started after 2 weeks' therapy. However, continuous observation during a 5-[27] or 7-day regimen[28] of ampicillin also did not reveal any substantial effect on steroid metabolism. Similar results have been obtained with erythromycin or trimethoprim in human subjects.[27] At present, no convincing evidence exists that antibiotics substantially interfere with oral contraceptive steroids at the level of the enterohepatic circulation. However, other mechanisms, such as interference with steroid-degradating enzymes in the liver, cannot be ruled out; these will be dealt with separately.

Use of antacids for several specific and nonspecific gastrointestinal complaints is widespread. Self-administration of antacids is a common phenomenon. Antacids are known to interact with several therapeutic agents, thereby reducing their bioavailability.[32] In vitro adsorption of synthetic sex steroids on antacid magnesium trisilicate has been demonstrated.[33] However, in vivo bioavailability of contraceptive steroids was unaffected by concomitant antacid administration. In 12 women who were given an antacid (magnesium trisilicate, 0.5 gm, and dried aluminium hydroxide, 0.25 gm) and a contraceptive pill (containing ethinyl estradiol, 30 μg, in combination with either norethisterone-acetate, 1 mg, or levonorgestrel, 150 μg) simultaneously, no change in the total bioavailability of any steroid was seen.[34] Moreover, no delay in the time taken to reach peak concentration was seen. The authors conclude that clinically significant interactions between oral contraceptives and antacids appear to be unlikely in the in vivo situation.

Mestranol is less commonly used as the estrogenic component of contraceptive steroid formulations. In the body, mestranol is rapidly converted to ethinyl estradiol by hydrolysis,[15] and the estrogenic effect is almost entirely caused by the demethylated compound. Mestranol and ethinyl estradiol can be regarded as equipotent.[35] After demethylation, mestranol is metabolized in a fashion similar to that with ethinyl estradiol, including enterohepatic recirculation.

The most commonly used progestogens in oral contraceptives are norethisterone, levonorgestrel, desogestrel, and less commonly, the pregnane derivatives cyproterone acetate and chlormadinone acetate. The progestogens norethynodrel, ethynodiol diacetate, lynestrenol, and norethisterone acetate are metabolized to norethisterone and become active as such. Desogestrel has to be converted to its active compound, 3-keto-desogestrel, and norgestimate is active after conversion to norgestrel-17β-acetate and levonorgestrel.

All of the steroids are well absorbed in human subjects, with levonorgestrel being completely bioavailable and norethisterone 70% available on average.

Norethisterone is chiefly metabolized by saturation of the double bond and reduction of the 3-keto group to a hydroxyl group. The metabolic pathways of levonorgestrel include 2α hydroxylation, 3α-5β reduction, 16β hydroxylation, glucuronidation of the 16β-hydroxy metabolite, and direct sulfate conjugation.[10] It is apparent that the progestogens have to be reduced at the 3 position before conjugation. Because little evidence exists for direct conjugation at the 17 position, the progestogens do not undergo enterohepatic recirculation as unchanged drugs. Any disruption of this latter mechanism would therefore have no impact on contraceptive efficacy.

Chronic inflammatory bowel disease and oral contraceptive steroids

Ample evidence exists of an association between chronic inflammatory bowel disease and smoking habits. Patients with ulcerative colitis tend to be nonsmokers,[36-40] and patients with Crohn disease tend to be

smokers.[36, 41] However, evidence also exists that women taking oral contraceptive steroids have an increased risk of Crohn disease.[42-44] According to Vessey et al.,[45] the use of oral contraceptives is more strongly associated with ulcerative colitis than with Crohn disease. A direct correlation between the duration of oral contraceptive use and the relative risk of Crohn disease has been demonstrated.[46] Accordingly, the relative risk is increased eight-fold after contraceptive pill use for more than 5 years; this high risk is normalized when at least 4 years have elapsed since the last pill use.

The pathogenetic role of oral contraceptives in chronic inflammatory bowel disease, if any exists, remains obscure. The observation that colonic Crohn disease is particularly associated with the use of contraceptive steroids suggests a vascular basis for the lesions. Kirsner and Shorter[47] proposed a role for transient insults, including ischemia, in the development of inflammatory bowel disease. Acute segmental colitis associated with pill use have been interpreted by ischemic episodes[48, 49] including the mesentery.[50-52]

The belief has been widespread that women who take oral contraceptive steroids and who have diarrhea are at risk of contraceptive failure. Women with an ileostomy might be at even higher risk because of an enhanced intestinal passage of gastrointestinal contents as well as the loss of enterohepatic steroid recirculation. In patients having undergone ileostomy for ulcerative colitis involving only the colon, the bioavailability of ethinyl estradiol and levonorgestrel (after a single oral dose of 50 μg of ethinyl estradiol and 250 μg of levonorgestrel) was not different from that of control subjects.[53] Similar results were obtained in patients with unoperated ulcerative colitis.[54] However, plasma levonorgestrel levels were reduced after either conventional ileostomy or proctocolectomy with construction of a continent ileostomy. The lowest levels of levonorgestrel were recorded in patients with the largest (10 and 35 cm) bowel resections. It is suggestive to explain these results by a reduced overall absorptive capacity caused by the loss of 35 to 45 cm of terminal ileum needed for construction of the pouch. However, direct administration of levonorgestrel into the pouch showed that the ileal mucosa had retained its absorptive capacity in spite of morphologic changes[55] and bacterial overgrowth.[56] The exact mechanism leading to reduced bioavailability under these conditions remains obscure.

Jejunoileal bypass has long been standard surgical procedure in the treatment of morbid obesity. The rationale for this operation is to bypass the major part of the small bowel to reduce its absorptive capacity.

In seven morbidly obese women, jejunoileostomy end side had been performed, leaving 37 cm of jejunum and 12.5 cm of ileum in continuity. After an oral load with either 3 mg of norethisterone or 250 μg of levonorgestrel, a significantly reduced bioavailability of either steroid was observed.[57] These data clearly demonstrate that oral contraceptive steroids are mainly absorbed in the small bowel and that contraceptive efficacy is dependent on its absorptive capacity. In case of small bowel diarrhea, contraceptive failure is likely to occur. In most instances, however, diarrhea is of colonic origin and should therefore not affect contraceptive safety.

REFERENCES

1. Population Reports. Oral contraceptives. Baltimore: John Hopkins University 1982; Series 6:191.
2. Back DJ, Breckenridge AM, Crawford FE, et al. An investigation of the pharmacokinetics of ethynylestradiol in women using radioimmunoassay. Contraception 1979;20: 263-73.
3. de la Pena A, Chenault CB, Goldzieher J. Radioimmunoassay of unconjugated plasma ethynylestradiol in women given a single dose of ethinylestradiol or mestranol. Steroids 1975;25:773-80.
4. Düsterberg R, Hümpel M, Wendt H. Plasma levels of active ingredients after single and repeated administration of a new oral contraceptive containing 2 mg of cyproterone acetate and 50 μg of ethinylestradiol (Diane) to five young women. Acta Obstet Gynecol Scand (Suppl) 1979;88:27-31.
5. Goldzieher JW, Dozier TS, de la Pena A, et al. Plasma levels and pharmacokinetics of ethinylestrogens in various populations. Contraception 1980;21:1-16.
6. Nilsson S, Nygren KG, Johansson EDB. Ethinyl estradiol in human milk and plasma after oral administration. Contraception 1978;17:131-9.
7. Pasqualini JR, Castallet R, Portois MC, Hill JL, Kincl FA. Plasma concentrations of ethinyl estradiol and norethindrone after oral administration to women. J Reprod Fertil 1977;49:189-93.
8. Speck U, Wendt H, Schulze PE, Jentsch D. Bioavailability and pharmacokinetics of cyproterone acetate-^{14}C and ethinylestradiol-^{3}H after oral administration as a coated tablet. Contraception 1976;14:151-63.
9. Warren RJ, Fotherby K. Plasma levels of ethinylestradiol after administration of ethinylestradiol or mestranol to human subjects. J Endocrinol 1973;59:369-70.
10. Orme M L'E, Back DJ, Breckenridge AM. Clinical pharmacokinetics of oral contraceptive steroids. Clin Pharmacokinet 1983;8:95-136.
11. Back DJ, Bates M, Burke MW, et al. Plasma Xa inhibitory activities and plasma concentrations of norgestrel and ethinyloestradiol in women on oral contraceptive steroids. Br J Clin Pharmacol 1979;8:505-6.
12. Hümpel M, Nieuweboer B, Wendt H, Speck U. Investigations of pharmacokinetics of ethinyloestradiol to specific consideration of a possible first-pass effect in women. Contraception 1979;19:421-32.
13. Back DJ, Bates M, Breckenridge AM, et al. Drug metabolism by gastrointestinal mucosa—clinical aspects. In: Prescott and Nimmo, eds. Drug absorption, Sydney, Australia: ADIS Press, 1980:80-7.
14. Back DJ, Breckenridge AM, MacIver M, et al. The gut wall metabolism of ethinyloestradiol and its contribution to the pre-systemic metabolism of ethinyloestradiol in humans. Br J Clin Pharmacol 1982;13:325-30.
15. Bolt HM, Bolt WH. Pharmacokinetics of mestranol in man in relation to its oestrogenic activity. Eur J Clin Pharmacol 1974;7:295-305.
16. Bolt HM, Bolt M, Kappus H. Interaction of rifampicin treatment with pharmacokinetics and metabolism of ethinylestradiol in man. Acta Endocrinol 1977;85:189-97.

17. Bolt HM, Kappus H, Dasbohrer R. Metabolism of 17α-ethinylestradiol by human liver microsomes in vitro: aromatic hydroxylation and irreversible protein binding of metabolites. J Clin Endocrinol Metab 1974;39:1072-80.
18. Williams JG, Longcope E, Williams KIH. Metabolism of [4-^{3}H] and [4-^{14}C] ethinylestradiol-3 methyl ether (mestranol) by women. Steroids 1975;25:343-54.
19. Helton ED, Williams MC, Goldzieher JW. Human urinary and liver conjugates of 17α-ethinyloestradiol. Steroids 1976;27:851-67.
20. Fotherby K. Metabolism of synthetic steroids by animals and man. In: Briggs and Diczfalusy, eds. Pharmacological models in contraceptive development. Stockholm, Sweden: WHO, 1974:119-48.
21. Cargill DI, Steinetz BG, Gosnell E, et al. Fate of ingested radio labelled ethinylestradiol and its 3-cyclopentyl ether in patients with bile fistulas. J Clin Endocrinol Metab 1969;29:1051-61.
22. Järvenpää P, Kosunen T, Fotsis T, Adlercreutz H. In vitro metabolism of estrogens by isolated intestinal microorganisms and by human faecal microflore. J Steroid Biochem 1980;13:345-9.
23. Back DJ, Breckenridge AM, Crawford FE, et al. The effects of rifampicin on the pharmacokinetics of ethinylestradiol in women. Contraception 1980;21:135-43.
24. Bacon JF, Shenfield GM. Pregnancy attribute to interaction between tetracycline and oral contraceptives. Br Med J 1980;1:293.
25. Dossetor J. Drug interactions with oral contraceptives. Br Med J 1975;4:467-8.
26. von Hempel E, Böhm W, Carol W, Klinger W. Medikamentöse Enzyminduktion und hormonale Kontrazeption. Zentralbl Gynäkol 1973;95:1451-7.
27. Adlecreutz H, Pulkkinen MO, Hämäläinen EK, Korpela JT. Studies on the role of intestinal bacteria in metabolism of synthetic and natural steroid hormones. J Steroid Biochem 1984;20:217-29.
28. Back DJ, Breckenridge AM, MacIver M, et al. The effects of ampicillin on oral contraceptive steroids in women. Br J Clin Pharmacol 1982;14:43-8.
29. Friedman CI, Huneke AL, Kim MH, Powell J. The effect of ampicillin on oral contraceptive effectiveness. Obstet Gynecol 1980;55:33-7.
30. Joshi JV, Joshi UM, Sankhali GM, et al. A study of interaction of low dose combination oral contraceptive with ampicillin and metronidazole. Contraception 1980;22:643-52.
31. Leigh DA, Reeves DS, Simmons K, Thomas AL, Wilkinson PJ. Talampicillin: a new derivative of ampicillin. Br Med J 1976;2:1378-80.
32. Hurwitz A. Antacid therapy and drug kinetics. Clin Pharmacokinet 1977;2:269.
33. Khalil SAH, Iwunagwu M. The in vitro uptake of some oral contraceptive steroids by magnesium trisilicate. J Pharm Pharmacol 1976;28(suppl):47.
34. Joshi JV, Sankolli GM, Shah RS, Joshi UM. Antacid does not reduce the bioavailability of oral contraceptive steroids in women. Int J Clin Pharmacol Ther Toxicol 1986;24:192-5.
35. Goldzieher JW, de la Pena A, Chenault CB, Wouterz TB. Comparative studies of ethinylestrogens used in oral contraceptives. II. Anti-ovulatory potency. Am J Obstet Gynecol 1975;122:619-24.
36. Benoni C, Nilsson A. Smoking habits in patients with inflammatory bowel disease. Scand J Gastroenterol 1984;19:824-30.
37. Bures J, Fixa B, Komarkova O, Fingerland A. Non-smoking: a feature of ulcerative colitis. Br Med J 1982;285:440.
38. Harries AD, Baird A, Rhodes J. Non-smoking: a feature of ulcerative colitis. Br Med J 1982;284:706.
39. Jick H, Walker AM. Cigarette smoking and ulcerative colitis. N Engl J Med 1983;308:261-3.
40. Logan FRA, Edmond M, Somerville KW, Langman MJS. Smoking and ulcerative colitis. Br Med J 1984;288:751-3.
41. Somerville KW, Logan RFA, Edmond M, Langman MJS. Smoking and Crohn's disease. Br Med J 1984;289:954-6.
42. Ramcharan S, ed. The Walnut Creek Contraceptive Drug Study: a prospective study of the side effects of oral contraceptives. Vol III. Washington, D.C.: US Government Printing Office, 1981.
43. Rhodes JM, Cockel R, Allan RN, et al. Colonic Crohn's disease and use of oral contraception. Br Med J 1984;288:595-6.
44. Royal College of General Practitioners: Oral contraceptives and health. London, England: Pitman Medical, 1974.
45. Vessey M, Jewell D, Smith A, Yeates D, McPherson K. Chronic inflammatory bowel disease, cigarette smoking, and use of oral contraceptives: findings in a large cohort study of women of childbearing age. Br Med J 1986;292:1101-3.
46. Lesko SM, Kaufman DW, Rosenberg L, et al. Evidence for an increased risk of Crohn's disease in oral contraceptive users. Gastroenterology 1985;89:1046-9.
47. Kirsner JB, Shorter RG. Recent developments in "nonspecific" inflammatory bowel disease (part two). N Engl J Med 1982;306:837-48.
48. Cotton PB, Lea Thomas M. Ischaemic colitis and the contraceptive pill. Br Med J 1971;3:27-8.
49. Kilpatrick ZM, Silverman JF, Betancourt E, Farman J, Lawson JP. Vascular occlusion of the colon and oral contraceptives. N Engl J Med 1968;278:438-40.
50. Anonymous: Small bowel ischemia and the contraceptive pill. Br Med J 1978;1:4.
51. Hurwitz RL, Martin AJ, Grossman BE, Waddell WR. Oral contraceptives and gastrointestinal disorders. Ann Surg 1970;172:892-6.
52. Ritschard TH, Filippini L. Entzündliche Darmerkrankungen und hormonelle Antikonzeption. Schweiz med Wschr 1986;116:594-7.
53. Grimmer SFM, Back DJ, Orme M L'E, Cowie A, Gilmore I, Tjia J. The bioavailability of ethinyloestradiol and levonorgestrel in patients with an ileostomy. Contraception 1986;33:51-9.
54. Nilsson LO, Victor A, Kral JG, Johansson EDB, Kock NG. Absorption of an oral contraceptive gestagen in ulcerative colitis before and after proctocolectomy and construction of a continent ileostomy. Contraception 1985;31:195-204.
55. Philipson BM, Brandberg A, Jagenburg R, Kock NG, Lager I, Ahren C. Mucosal morphology, bacteriology, and absorption in intraabdominal ileostomy reservoir. Scand J Gastroenterol 1975;10:145-53.
56. Kay RM, Cohen Z, Siu KP, Petrunka CN, Strasberg SM. Ileal excretion and bacterial modification of bile acids and cholesterol in patients with continent ileostomy. Gut 1979;21:128-32.
57. Victor A, Odlind V, Kral JG. Oral contraceptive absorption and sex hormone binding globulins in obese women: effects of jejunoileal bypass. Gastroenterol Clin North Am 1987;16:483-91.

Influence of oral contraceptives on drug therapy

Alexander T. Teichmann, Prof. Dr. med.
Göttingen, West Germany

Interferences between drugs and oral contraceptives are considered to alter pharmacokinetics and thus the efficacy of steroidal hormones. It should be noted, however, that steroids can also modify the metabolism and pharmakodynamic effects of various substances. To the present knowledge, phase I (i.e., oxidation, demethylation) and phase II reactions (conjugation) are concerned. Drugs sharing those enzymatic systems with oral contraceptives experience either an increase in bioavailability by inhibition of oxidative metabolism or undergo accelerated elimination by induced conjugation. Such interaction may be of practical interest in subjects who take oral contraceptives and are simultaneously treated with antidepressants, antihypertensives, insulin, synthetic glucocorticoids, theophylline, and caffeine. (Am J Obstet Gynecol 1990;163:2208-13.)

Key words: Oral contraceptives, drug interactions, pharmacokinetics, pharmacodynamics

Although oral contraceptives (OC) are among the most widely used drugs in the world whose effects and side effects have been extensively investigated, their pharmacology still remains a perplexing field of multivariate processes. The biochemical and physiologic actions of estrogens and gestagens are not simply additive when given in combination, and their interactions with other drugs lead to an even greater degree of complexity. The following attempt to review the effects of OCs on the pharmacology of other drugs will inevitably be all but satisfactory not only because it is made by a gynecologist but also because a reviewer's task is to condense and stratify information rather than to present all details available.

Three major principles of drug interactions exist, of which the chemical reaction between drugs is unlikely to apply to steroid hormones. OCs can, however, influence pharmacokinetics, particularly through protein binding patterns, and metabolism, and they can also interfere with pharmacodynamics.

Most commonly interactions of OCs with other drugs will take place if phase I reactions are concerned. It is well known that cytochrome systems P-450 and P-448 can be inhibited by OCs either directly or by competition. Moreover, phase II reactions, namely, conjugation with acids, are positively influenced by OCs through enzyme induction. Orally administered ethinyl estradiol (EE) exerts a strong stimulatory effect on protein synthesis, which is partly opposed by gestagens. Finally, receptor-mediated hormonal actions may interfere with wanted or unwanted drug effects of hormonal and nonhormonal agents.

From the Department of Obstetrics and Gynecology, Georg-August-Universität Göttingen.
Reprint requests: Prof. Dr. med. A.T. Teichmann, Univ.-Frauenklinik Göttingen, Robert-Koch-Straße 40, 3400 Göttingen, West Germany.
6/0/24557

Before I discuss groups of commonly prescribed pharmaceutical products in detail with respect to the interfering actions of OCs, a few basic remarks are necessary:

1. Combined OCs represent a homogenous group of hormonal agents as far as the type of estrogen and the oral route of administration are concerned.
2. Combined OCs are heterogenous with regard to the doses of EE and the type and dosage of the gestagen.
3. The biologic effects of OCs do not depend solely on their composition and route of administration, but to a large extent depend on individual factors such as age (metabolic activity), individual pharmacokinetics, dietary habits, body mass, physical activity, neuroendocrinologic conditions, and numerous others.

These characteristics of OCs and their actions in individual organisms will be largely omitted in the following summary of our knowledge on how OCs modify the effects of other drugs. Although I am quite aware that simplifications may sometimes lead to wrong conclusions, limited space demands this sacrifice for a more pragmatic approach (Table I). Contradictory findings in the following studies cited may be explained by factors such as differences in OC composition and individual conditions.

Analgesics belong to the most frequently taken drugs available without medical prescription, and thus it is not remarkable that they are often taken during hormonal contraception. Phenazone is mainly metabolized by oxidation. Its halflife is prolonged, and its clearance rate is reduced by OCs as a result of delayed hydroxylation. Similar conclusions were reached by the authors of other studies investigating the effects of EE on pethidine, a synthetic morphine-analog. Morphine undergoes conjugation with glucaronic acid, and enzy-

Table I. Influence of OCs on the pharmakinetics of commonly prescribed drugs

Type	*Generic*	*Bioavailability*		*Authors*
		Augmented	*Decreased*	
Analgesics	Phenazone	Inhibited oxidation		Teunissen et al., 1982[28]
		Decreased clearance		Abernethy et al., 1981[29]
		Increased half-life		Crawford et al., 1966[30]
	Aminophenazone	Inhibited demethylation		Herz et al., 1977[31]
	Paracetamol		Induced conjugation	Mitchel et al., 1983[3]
				Miners et al., 1983a
			Induced oxidation	Miners et al., 1983
			Increased clearance	Abernethy et al., 1982[32]
	Aspirin		Induced conjugation	Miners et al., 1986[2]
	Morphine		Induced conjugation	Mitchell et al., 1983b
	Ethylmorphine	Inhibited demethylation		Carter et al., 1973[1]
	Pethidine	Inhibited oxidation		Herz et al., 1977[31]
Tranquilizers	Alprazolam			Stoehr et al., 1984[6]
	Trizolam (bromazepam)			Stoehr et al., 1984[6]
				Ochs et al., 1987[33]
	Chlordiazepoxid	Decreased clearance		Roberts et al., 1979[34]
				Greenblatt et al., 1977[35]
				Patwardhan et al., 1983[36]
	Nitrazepam	Inhibited oxidation		Jochemsen et al., 1982[37]
	Diazepam			Giles et al., 1981[38]
				Abernethy et al., 1982, 1984[10,32]
				Routledge et al., 1981[39]
				Greenblatt et al., 1980[40]
				Stoehr et al., 1984[6]
	Lorazepam		Increased clearance	Patwardhan et al., 1983[36]
	Temkzepam			
	Oxazepam		(Increased conjugation)	Stoehr et al., 1984[6]
				Patwardhan et al., 1983[36]
				Abernethy et al., 1983[41]
Tricyclic antidepressants	Imipramine	Decreased clearance		Abernethy et al., 1984[10]
Antiepileptics	Phenytoin	Inhibited oxidation		Kutt et al., 1968[42]
				De Leacy et al., 1979[43]
				Hooper et al., 1974[44]
Barbiturates		Inhibited oxidation		Tephly and Mannering, 1969[45]
Anticoagulants	Coumarin derivatives	Inhibited oxidation		De Teresa et al., 1979[46]
				MacLeod and Sellers, 1976[47]
				Koch-Weser et al., 1971[48]
Antihypertensives	Metoprolol			
	Oxprenolol	Inhibited oxidation		Kendall et al., 1982[49]
	Propranolol			Kendall et al., 1984[50]
Lipid-lowering drugs	Clofibrate		Increased conjugation	Miners et al., 1984[51]
			Increased glucuronidation	
Glucocorticoids	Cortisol	Decreased clearance		Marks et al., 1961[52]
	Prednisolone	Decreased clearance (inhibited oxidation)		Gustavson, 1986[53]
				Legler and Benet, 1986[54]
				Shaw et al., 1983[55]
				Boekenoogen et al., 1983[56]
				Kozower et al., 1974[57]
				Frey et al., 1984[58]
				Frey and Frey, 1985[59]
Markers	Theophylline			Gardner and Jusko, 1986[15]
	Aminophylline	Inhibited oxidation		Roberts et al., 1983[17]
	Oxitriphylline			Gardner et al., 1983[60]
	Coffeine	Inhibited oxidation		Pathwardhan et al., 1980[61]
				Abernethy et al., 1985[62]
				Meyer et al., 1988[63]

matic process that is stimulated by OCs, leading to enhanced metabolism and thus inactivation of this drug. In rat liver preparations, however, as shown by Carter et al.,[1] ethylmorphine metabolism is reduced by inhibition of its demethylation in the presence of OCs. The ability of OCs to induce enzymatic conjugation, namely, with glucuronic and sulfuric acid, seems to be the mode of action by which the metabolism of paracetamol and

aspirin is enhanced. In particular, glucuronidation accounts for 90% of the biotransformation of paracetamol. Because this substance is also metabolized by oxidation, the corresponding influence of OCs on this reaction is of interest. However, the findings reported by Miners et al.[2] and Mitchell et al.[3] are contradictory. It may be of importance to note that Gupta et al.[4] found the influence of OCs on aspirin glucuronidation to decrease with the time of OC use. Morphine shows decreased bioavailability as a result of stimulatory effect on conjugation with glucuronic acid, whereas in rat liver preparation, the demethylation of ethylmorphine seems to be inhibited. Pethidine is negatively influenced in its metabolism by inhibition of oxidation.

Whereas numerous reports deal with contraceptive efficacy that is threatened by antibiotics and tuberculostatics, namely, rifampicin, little is known about the effects of OCs on antibiotic treatment. Only in the case of ampicillin were no alterations in plasma levels found in pilltakers between days 21 and 28 of the artificial cycle.

Like analgesics, tranquilizers, namely, the benzodiazepine, are among the most frequently used drugs in the Western population. Most of these drugs undergo oxidation, and only a few are metabolized predominantly by conjugation. Analogous to cimethidine, a strong inhibitor of microsomal oxidation, OCs inhibit or at least slow down oxidation and thus lead to a reduction in the clearance rates of the benzodiazepines. Because the activity of microsomal oxidation processes decrease with age and because female sex hormones negatively influence the cytochrome P-450 and P-448 systems, both factors diminish clearance rates of the first group of tranquilizers.

In contrast, those benzodiazepines that undergo conjugation as their main metabolic pathway experience a higher clearance rate in OC-treated subjects. Other benzodiazepines, such as clotiazepam and triazolam, showed no pharmacologic changes from OCs.[5, 6]

For the practical clinician, the question arises whether and to what extent pharmacokinetic interactions between OCs and benzodiazepines correspond to clinical interactions. A significant delay in psychomotor impairment of OC users compared with nonusers, which was observed by Ellinwood et al.[7] did not show any correlation with the plasma levels of diazepam, which had been found to be elevated in women and OC users compared with men. Contrasting findings were reported by Krobath et al.[8] for alprazolam, triazolam, and lorazepam. Psychomotor changes were most marked in women who were on the pill. However, again plasma levels did not correlate with the observed clinical effect. Therefore, other hypothetic pharmacodynamic interferences must be postulated, such as a direct change in membrane properties, to explain the lack of clinical manifestation of pharmacokinetic findings.

Tricyclic antidepressants, such as imipramine, clomipramine, and chlorpromazine, were suspected of having increased side effects in OC users on the basis of the observations by Prange.[9] It has been demonstrated that the clearance rate of imipramine is negatively influenced by OCs whereas protein binding and half-life are unaffected. Imipramine is metabolized by demethylation and oxidation before conjugation with glucuronic acid; OCs seem to inhibit oxidation and thus augment bioavailability from 27% to 44%.[10-12] In contrast, clomipramine is not influenced by OCs, although its metabolic rate is enhanced by estrogens and inhibited by gestagens, at least in animal models.[13] The psychotropic effects of sex steroids must be added to the pharmacokinetic interferences. Thus it seems wise to monitor the clinical effects of antidepressants thoroughly if they are given to OC users.

Antiepileptics of the phenytoin type as well as barbiturates are metabolized by oxidation. In addition to influencing the protein binding of phenytoin, OCs lead to a lower oxidation rate in both groups. Since both estrogens and gestagens interfere with amine metabolism in the brain and are known to augment (estrogens) or lower (gestagens) convulsibility, various pharmacodynamic effects are assumed to overlap the clinical manifestations of altered pharmacokinetics.

Indirectly acting anticoagulants, vitamin K antagonists, or coumarin derivatives undergo enzymatic oxidation that is inhibited by cimethidine and induced by phenobarbital. Studies conducted in OC users showed the inhibiting effects of OCs. Moreover, pharmacodynamic interactions may well be of practical importance. Coumarins inhibit the synthesis of vitamin K–dependent coagulation factors VII, IX, and X. Estrogens, namely, orally administered EE, stimulate various coagulation and fibrinolytic agents, in particular factors VII and X, and thus lead to a partial functional antagonism. The net effect of pharmacokinetic and dynamic interferences depends not only on EE dose but also on the type and dose of the gestagen. Because the influence of vitamin K antagonists is the limiting factor in anticoagulatory therapy, OCs clearly weaken their effect.

The potency of OCs to induce or exaggerate elevated blood pressure is well known. EE and gestagens, with the exception of progesterone, cause water and sodium retention and act by way of antidiuretic hormone and aldosterone. The predominant influence is that of EE on the synthesis of renin substrate, thus stimulating the renin angiotensin aldosterone system. Other sites of action of estrogens may be in the heart muscle and the vessel wall. Antihypertensive drugs show great het-

erogenity in their chemical structure and modes of action. Because OCs influences blood pressure regulation, pharmacodynamic interactions are to be expected, particularly if pill-induced forms of hypertension are concerned.

β-Receptor blockers, such as metroprolol, oxprenolol, and propranolol, undergo oxidation that is inhibited by OCs, leading to higher bioavailability. Acebutolol kinetics do not interfere with OCs since this drug undergoes unmetabolized renal elimination. The interaction of an OC with centrally acting antihypertensives such as clonidine may be mediated by α-adrenergic and dopaminergic receptors. Pharmacokinetic interactions are not known.[14]

Clofibrate is commonly used in subjects with hyperlipidemia. Its glucuronidation rates are elevated by OCs, leading to a 48% increase in the clearance rate. Thus the pharmacologic effect of clofibrate is weakened. However, the well-known pharmacodynamic influence of estrogens and gestagens on lipid and lipoprotein metabolism should not be neglected. It largely depends on the type of contraceptive pill and on a great variety of pretherapeutic conditions that have been mentioned above. A general statement that subjects treated for hyperlipidemia should not receive OCs cannot be made at this time on the basis of pharmacokinetic and pharmacodynamic interferences. It is still not clear which OCs have effects on the different forms of hyperlipidemia, what these effects are, and how treatment results are modified by OCs.

In contrast to lipid and lipoprotein metabolism, the pharmacodynamic influences of OCs mainly relate to gestagen interference with *antidiabetic* treatment. Gestagen-induced peripheral insulin resistance may lead in some cases to dose adaptation of insulin. Oral antidiabetic drugs are not likely to be prescribed to juvenile diabetics, which may account for the vast majority of diabetic patients being on the pill.

Of the various hormonal treatment regimens, glucocorticoids are among the most frequently prescribed drugs. It is well known that cortisone-binding globulin and the levels of endogenous cortisol are elevated by EE. Since Spangler et al.[15] reported a two-fold to 20-fold increase in the antiinflammatory effects of topical hydrocortisone on OC users, it has been established that estrogens may well influence the ratio of free and protein-bound glucocorticoids. The mechanism of estrogen action as described for prednisolone seems not only to act by altered protein-binding patterns between cortisol-binding globulin and albumin but also by an inhibited metabolism of prednisolone, mainly because of reduced microsomal oxidation. No influences on pharmacokinetics have been found for fluocortolone.

Substances that are characteristic for specific metabolic processes may be regarded as "markers," in addition to their therapeutic properties. Such markers are theophylline and derivatives, caffeine, and alcohol.

Theophylline undergoes oxidation by cytochrome P-450 and P-448–dependent systems. Both oxidation and clearance rates are lowered by OCs. Because smoking exerts an enzyme-inductive effect, inhibitory actions of OCs on theophylline metabolism are not observed in smoking subjects who are on the pill. The enzymatic and metabolic link between OC and tobacco action, however, remains uncertain.[16, 17]

Caffeine metabolism is mediated by cytochrome P-440 oxidation. As with theophylline, the clearance rates for caffeine are negatively influenced by OCs.

The findings concerning alcohol and alcohol dehydrogenase activity and interaction with OCs are not clearly defined. Decreased elimination rates are not likely to result from OC-induced cytochrome P-450 inhibition, to which only a minor metabolic fraction is subjected. Clinically relevant conclusions cannot be drawn at this time.[18, 19]

OCs exert influences on various vitamins and minerals. Increased plasma levels of iron, copper, and vitamins A, D, and K were found in OC users, whereas concentrations of zinc, folic acid, and vitamins B_1, B_6, B_{12} and C decreased with the use of estrogens and gestagens.[20-27] Whereas in subjects with normal dietary habits these effects will not be clinical relevant, under deprived conditions, reduced plasma levels of essential factors may be of some concern.

In conclusion, as noted above, the effects of oral contraceptives on drug therapy are related mainly to the inhibition of microsomal oxidation and demethylation, as well as the induction of enzymes involved in conjugation reactions. Drugs that share these metabolic pathways will be affected in their pharmacokinetics.

However, various pharmacodynamic interferences between OCs and the effects of other drugs may, in most instances, be counterbalanced with kinetic alterations, resulting in a weak or no obvious clinical manifestations. Attention should be paid mainly to a possible modification of the pharmacologic effects in treatment with antidepressants antihypertensives, insulin, synthetic glucocorticoids, theophylline, and caffeine. Even if such interactions may not be present in all subjects, clinical consequences must be drawn, particularly in predisposed persons.

REFERENCES

1. Carter DE, Goldman JM, Bressler R, Huxtable RJ, Christian CD, Heine MW. Effects of oral contraceptives on drug metabolism. Clin Pharmacol Ther 1973;15:22-31.
2. Miners JO, Grgurinovich N, Whitehead AG, Robson RA, Birket DJ. Influence of gender and oral contraceptive

steroids on the metabolism of salicylic acid and acetylsalicylic acid. Br J Clin Pharmacol 1986;22:135-42.
3. Mitchell MC, Hanew T, Meredith CG, Schenker S. Effects of oral contraceptive steroids an acetaminophen metabolism and elimination. Clin Pharmacol Ther 1983;34:48-53.
4. Gupta KC, Joshi JV, Hazari K, Pohujani SM, Satoskar RS. Effect of low dose estrogen combination oral contraceptive on metabolism of aspirin and phenylbutazone. Int J Pharmacol Ther Toxicol 1982;20:511-3.
5. Parke DV. Induction of drug metabolising enzymes. In: Parke DV, ed. Enzyme induction. London: Plenum Press, 1984:228.
6. Stoehr GP, Kroboth PD, Juhl RP, Wender DB, Phillips JP, Smith RB. Effect of oral contraceptives on triazolam, temazepam, alprazolam, and lorazepam kinetics. Clin Pharmacol Ther 1984;36:683-90.
7. Ellinwood EH Jr, Easler ME, Linnoila M, Molter DW, Heatherly DG, Bjornsson TD. Effects of oral contraceptives on diazepam-induced psychomotor impairment. Clin Pharmacol Ther 1984;35:360-6.
8. Kroboth PD, Smith RB, Stoehr GP, Juhl RP. Pharmacodynamic evaluation of the benzodiazepine—oral contraceptive interaction. Clin Pharmacol Ther 1985;38:525-32.
9. Prange AJ. Estrogen may well affect the response to antidepressants. JAMA 1972;219:143-4.
10. Abernethy DR, Greenblatt DJ, Shader RI. Imipramine disposition in users of oral contraceptive steroids. Clin Pharmacol Ther 1984;35:792-7.
11. Gringas M, Beaumont G, Grieve A. Clomipramin and oral contraceptives: an interaction study—clinical findings. J Int Med Res 1980;8:76-80.
12. Luscombe DK, John V. Influence of age, cigarette smoking and the oral contraceptive on plasma concentrations of clomipramin. Postgrad Med J 1980;56:99-102.
13. Fletcher HP, Miya TS, Bousquet WF. Influence of estradiol on the disposition of chlorpromazine in the rat. J Pharm Sci 1965;54:1007-9.
14. Chalmers JS, Fulli-Lemaire J, Cowen PJ. Effects of the contraceptive pill on sedative responses to clonidine and apomorphine in normal women. Psychol Med 1985; 15:63-367.
15. Spangler AS, Antoniadas HN, Sotman SL, Inderbitzin TM. Enhancement of the anti-inflammatory action of hydrocortisone by estrogen. J Clin Endocrinol 1969;29: 650-5.
16. Gardner MJ, Jusko WJ. Effects of oral contraceptives and tobacco use on the metabolic pathways of theophylline. Int J Pharmacol 1986;33:55-64.
17. Roberts RK, Grice J, McGuffie C, Heilbronn L. Oral contraceptive steroids impair the elimination of theophylline. J Lab Clin Med 1983;101:821-5.
18. Jones MK, Jones MB. Ethanol metabolism in women taking oral contraceptives. Alcohol Clin Exp Res 1984;8:8.
19. Hobbes J, Boutagy J, Shenfield GM. Interactions between ethanol and oral contraceptive steroids. Clin Pharmacol Ther 1985;38:371-80.
20. Webb JL. Nutritional effects of oral contraceptive use. A review. J Reprod Med 1980;25:150-6.
21. Schenker JG, Jungreis E,, Polishuk WZ. Oral contraceptives and correlation between copper and ceruloplasmin levels. Int J Fertil 1972;17:28-32.
22. Rhode BM, Cooper BA, Farmer FA. Effect of orange juice, folic acid and oral contraceptives on serum folate in women taking a folate-restricted diet. J Am Coll Nutr 1983;2:221-30.
23. Rivers JM, Devine MM. Plasma ascorbic acid concentrations and oral contraceptives. Am J Clin Nutr 1972; 25:684-9.
24. Luhby AL, Brin M, Gordon M, et al. Vitamin B_6 metabolism in users of oral contraceptive agents. I. Abnormal urinary xanthurenic acid excretion and its correction by pyridoxine. Am J Clin Nutr 1971;24:684-93.
25. Aly HE, Donald EA, Simpson MH. Oral contraceptives and vitamin B_6 metabolism. Am J Clin Nutr 1971;24:297-303.
26. Heilmann E. Orale Kontrazeptiva und Vitamine. Dtsch Med Wochenschr 1979;104:144-6.
27. Basu TK. Drug and vitamin interactions in adult. Int J Vitamin Nutr Res 1984;54:157-68.
28. Teunissen MWS, Srivastava AK, Breimer DD. Influence of sex and oral contraceptive steroids on antipyrine metabolite formation. Clin Pharmacol Ther 1982;32: 240-6.
29. Abernethy DR, Greenblatt DJ. Impairment of antipyrin metabolism by low-dose oral contraceptive steroids. Clin Pharmacol Ther 1981;29:106-10.
30. Crawford JS, Rudolfsky S. Some alterations in the pattern of drug metabolism associated with pregnancy, oral contraceptives and the newly born. Br J Aneasth 1966; 38:446-54.
31. Herz R, Koelz HR, Haemmerli MP, Benes J, Blum AL. Inhibition of hepatic demethylation of aminopyrine by oral contraceptive steroids in humans. Eur J Clin Invest 1978;8:27-30.
32. Abernethy DR, Greenblatt DJ, Divoll M, Arendt R, Ochs HR, Shader RI. Impairment of diazepam metabolism by low-dose estrogen-containing oral contraceptive steroids. N Engl J Med 1982;306:791-2.
33. Ochs HR, Greenblatt DJ, Friedman H, et al. Bromazepam pharmacokinetics: influence of age, gender, oral contraceptives, cimetidine and propranolol. Clin Pharmacol Ther 1987;41:562-70.
34. Roberts RK, Desmond PV, Wilkinson GR, Schenker S. Disposition of chlordiazepoxide: sex differences and effects of oral contraceptives. Clin Pharmacol Ther 1979; 25:826-31.
35. Greenblatt DJ, Shader RI, Franke K, MacLaughlin DS, Ransil BJ, Koch-Weser J. Kinetics of intravenous chlordiazepoxid: sex differences in drug distribution. Clin Pharmacol Ther 1977;22:893-903.
36. Patwardhan RV, Mitshell MC, Johnson RF, Schenker S. Differential effects of oral contraceptive steroids on the metabolism of benzodiazepines. Hepatology 1983;3:248-53.
37. Jochemsen R, van der Graaff M, Boeijinga JK, Breimer DD. Influence of sex, menstrual cycle and oral contraception on the disposition of nitrazepam. Br J Clin Pharmacol 1982;13:319-24.
38. Giles HG, Sellers EM, Naranjo CA, Frecker RC, Greenblatt DJ. Disposition of intravenous diazepam in young men and women. Eur J Clin Pharmacol 1981;20:207-13.
39. Routledge PA, Stargel WW, Kitchell BB, Barchowsky A, Shand DG. Sex-related differences in the plasma protein binding of lidocain and diazepam. Br J Clin Pharmacol 1981;11:245-50.
40. Greenblatt DJ, Allen MD, Harmatz JS, Shader RI. Diazepam disposition determinants. Clin Pharmacol Ther 1980;27:301-12.
41. Abernethy DR, Greenblatt DJ, Ochs HR, et al. Lorazepam and oxazepam kinetics in woman on lowdose oral contraceptives. Clin Pharmacol Ther 1983;33:628-32.
42. Kutt H, McDowell F. Management of epilepsy with diphenylhydantoin sodium. JAMA 1968;203:969-72.
43. de Leacy EA, McLeay CD, Eadie MJ, Tyrer JH. Effects of subjects' sex and intake of tobacco, alcohol and oral contraceptives on plasma phenytoin levels. Br J Clin Pharmacol 1979;8:33-6.
44. Hooper WD, Bochner F, Eadie MJ, Tyrer JH. Plasma protein binding of diphenylhydantoin. Effects of sex hormones, renal and hepatic disease. Clin Pharmacol Ther 1974;15:276-82.
45. Tephyly TR, Mannering GJ. Inhibition of drug metabolism by steroids. Med Pharmacol 1969;4:10-4.
46. de Teresa E, Vera A, Ortigosa J. Pulpon LA, Arus AP, de Artaza M. Interaction between anticoagulants and con-

traceptives: an unsuspected finding. Br Med J 1979; 2:1260-1.
47. MacLeod SM, Sellers EM. Pharmacodynamic and pharmacokinetic drug interactions with coumarin anticoagulants. Drugs 1976;11:461-70.
48. Koch-Weser J, Sellers EM. Drug interactions with coumarin anticoagulants. N Engl J Med 1971;285:487-98, 547-58.
49. Kendall MJ, Quarterman CP, Jack DB, Beeley L. Metoprolol pharmacokinetics and the oral contraceptive pill. Br J Clin Pharmacol 1982;14:120-2.
50. Kendall MJ, Jack DB, Quarterman CP, Smith SR, Zaman R. Beta-adrenoceptor blocker pharmacokinetics and the oral contraceptive pill. Br J Clin Pharmacol 1984;17:87S-9S.
51. Miners JO. Gender and oral contraceptive steroids as determinants of drug glucoronidation: effects on cloribric acid elimination. Br J Clin Pharmacol 1984;18:240-3.
52. Marks LJ, Benjamin G, Duncan FJ, O'Sullivan VI. Comparative effects of ethinyl estradiol, 17α-ethiyl-19-nortestosterone and methyl-testosterone on the plasma clearance of infused cortisol. J Clin Endocrinol 1961; 21:826-32.
53. Gustavson LE, Legler UF, Benet LZ. Impairment of prednisolone disposition in women taking oral contraceptives or conjugated estrogens. J Clin Endocrinol Metab 1986; 62:234-7.
54. Legler UF, Benet L. Marked alterations in dose-dependent prednisolone kinetics in women taking oral contraceptives. Clin Pharmacol Ther 1986;39:425-9.
55. Shaw MA, Back DJ, Aird SA, Grimmer SF, Orme MC. Urinary concentrations of steroid glucuronides in women taking oral contraceptives. Contraception 1983;28:69-75.
56. Boekenoogen SJ, Szefler SJ, Jusko WJ. Prednisolone disposition and protein binding in oral contraceptive users. J Clin Endocrinol Metab 1983;56:702-9.
57. Kozower M, Veatch L, Kaplan MM. Decrease clearance of prednisolone, a factor in the development of corticosteroid side effects. J Clin Endocrinol Metab 1974;38:407-12.
58. Frey BM, Schaad HJ, Frey FJ. Pharmacokinetic interaction of contraceptive steroids with prednisone and prednisolone. Eur J Clin Pharmacol 1984;26:505-11.
59. Frey BM, Frey FJ. The effect of altered prednisolone kinetics in patients with the nephrotic syndrome and women taking oral contraceptive steroids on human mixed lymphocyte cultures. J Clin Endocrinol Metab 1985;60:361-9.
60. Gardner MJ, Tornatore KM, Jusko WJ, Kanarkowski R. Effects of tobacco smoking and oral contraceptive use on theophylline disposition. Br J Clin Pharmacol 1983;16: 271-80.
61. Patwardhan RV, Desmond PV, Johnson RF, Schenker S. Impaired elimination of caffeine by oral contraceptive steroids. J Lab Clin Med 1980;95:603-8.
62. Abernethy DR, Todd EL. Impairment of caffeine clearance by chronic use of low-dose estrogen–containing oral contraceptives. Eur J Clin Pharmacol 1985;28:425-8.
63. Meyer FP, Canzler E, Giers H, Walther H. Langzeituntersuchung zum Einfluß von Non-Ovlon auf die Pharmakokinetik von Coffein im intraindividuellen Vergleich. Zent Bl Gynäkol 1988;110:1449-54.

Oral contraception in disease states

M. Breckwoldt, P. Wieacker, and F. Geisthövel

Freiburg im Breisgau, Germany

Oral contraceptives are clearly contraindicated in patients with a history of thromboembolic disease, ischemic heart attack, or cerebral stroke. Patients requiring long-term anticoagulant treatment can be treated with gonadotropin-releasing hormone analogs to prevent ovulation, because ruptured follicles can cause massive intraperitoneal bleeding. Patients with essential hypertension and severe liver diseases should also discontinue treatment 4 weeks before major elective surgery. Migraine and diabetes mellitus are regarded as relative contraindications, depending on the individual situation. Long-term diseases, such as Crohn's disease, epilepsy, and sickle cell anemia, also require individualized consultation. (Am J Obstet Gynecol 1990;163:2213-6.)

Key words: Oral contraceptives, thromboembolic disease, ischemic heart attacks, essential hypertension, severe liver disease, diabetes mellitus

From the Division of Clinical Endocrinology, Department of Obstetrics and Gynecology, University of Freiburg.
Reprint requests: Professor M. Breckwoldt, Department of Obstetrics and Gynecology, University of Freiburg, Hugstetterstrasse 55, D-7800 Freiburg im Breisgau, Germany.
6/0/25612

The use of oral contraceptives (OCs) in disease states and during drug therapy requires consideration of absolute and relative contraindications. This article will concentrate on the various forms of long-term diseases and discuss if the administration of OCs is justified for

Table I. Absolute contraindications

Arterial or venous thrombosis
Ischemic heart disease
Cerebral hemorrhage
Protein C and protein S deficiency
Antithrombin III deficiency
Hypertension
Hypercholesterolemia
Severe liver diseases
Estrogen-dependent breast cancer
Elective surgery

Table II. Relative contraindications

Migraine
Diabetes
Crohn's disease
Epilepsy
Sickle cell disease

each disease or if other alternative methods of contraception are preferred.

Absolute contraindications to OCs are listed in Table I. There is general agreement that OCs are clearly contraindicated in patients with a history of arterial or venous thrombosis, ischemic heart disease, cerebral hemorrhage, and stroke. Epidemiologic data provide evidence of an increased relative risk of venous thromboembolism in otherwise healthy OC users. No consensus has been reached for the risk of ischemic heart disease or cerebral stroke in OC users. Thromboembolic complications can be attributed to the fact that ethinyl estradiol can increase plasma levels of blood clotting factors (factors II, VII, and X) associated with a decrease in antithrombin III concentrations.[1-3] Consequently, patients with hypercoagulability caused by inherited defects of the clotting system, such as protein C, protein S, or antithrombin III deficiency, should be excluded from using OCs, because these patients tend to develop thromboembolic complications. Patients with a history of thromboembolic disease require long-term anticoagulant treatment. However, ovulation under these conditions can lead to massive intraperitoneal hemorrhage. In recent years, five patients receiving long-term warfarin treatment were admitted to our department in severe hemorrhagic shock. Abdominal surgery revealed heavy intraperitoneal bleedings from ruptured follicles or corpora lutea.[4] Pregnancies occurring during treatment with warfarin are of particular concern, because teratogenic effects have been described.[5] Therefore ovulation and pregnancy should be prevented in these patients. To suppress ovulation, these patients can be given a combined gonadotropin-releasing hormone analog/progesterone regimen.[4, 6] Low-dose treatment with gonadotropin-releasing hormone analogs (Buserelin), nasally applied, suppresses luteinizing hormone surges, whereas basal ovarian estrogen secretion is maintained. Analogs of gonadotropin-releasing hormone are devoid of undesirable metabolic side effects. Side effects associated with the use of gonadotropin-releasing hormone analogs are related only to estrogen deficiency. Constant endogenous estrogen secretion is sufficient to prevent loss of bone mass and to stimulate endometrial proliferation. To oppose continuous estrogen effects, vaginal progesterone suppositories are applied in a cyclic fashion to induce secretory transformation of the endometrium and consequently menstrual bleeding.[6] Progesterone is also free of unwanted metabolic side effects.

This approach to birth control is of course limited to a selected group of patients who are at high risk for thromboembolic complications and require careful monitoring. In more than 400 cycles, the pattern of menstrual bleeding was acceptable; only one pregnancy occurred.[6]

Hypertension is also regarded as a contraindication, because OCs can induce elevated blood pressure, presumably by stimulation of hepatic angiotensinogen production, leading to activation of the angiotensin-renin-aldosterone system.[7, 8] OC-induced hypertension is fully reversible after discontinuation of the drug. Patients with a blood pressure of 140/90 mm Hg should be excluded from the use of OCs, because these patients are at increased risk of having hemorrhagic stroke. Monitoring of blood pressure before the prescription of OCs and during OC intake is mandatory. Consequently, essential hypertension is considered an absolute contraindication. However, pregnancy-related hypertension does not correlate with OC intake in this regard.[9] Patients with a history of pregnancy-induced hypertension remain generally normotensive during OC intake. Gestagen-only pills or intrauterine devices can be recommended as alternative methods to prevent pregnancies in patients with essential hypertension.

Inherited hypercholesterolemia is also considered a contraindication to the use of OCs, because patients with chronically elevated cholesterol levels tend to develop arteriosclerosis and consequently hypertension.[10] These patients are also at increased risk for developing ischemic heart disease and myocardial infarction. However, the low-dose OCs presently used do not affect the lipoprotein pattern adversely; the high-density lipoprotein/low-density lipoprotein cholesterol ratio remains within normal limits. "The lower the steroid doses and the more balanced the ratio of progesterone to EE_2, the smaller the change in lipid levels from pretreatment values."[11] The prescription of OCs in these patients should be oriented according to vascular conditions.

Severe liver diseases, including porphyria and benign

liver tumors (adenomas), are contraindications to the use of OCs. Benign liver lesions can be induced by 17-acylated synthetic steroids in rare instances.[12] The incidence of benign liver tumors among OC users has been calculated in the order of 1:200,000.[12, 13] A causal relationship between the intake of OCs and the development of liver lesion is evidenced by the fact that these tumors regress significantly after discontinuation of the pill. We have followed three patients with benign liver tumors, which were probably induced by OCs. In all three patients the tumor regressed remarkably after discontinuation of the drug. The size of the tumor was followed by ultrasonography. Subsequently, these patients became pregnant. All three pregnancies were uneventful, the infants developed normally, and the size of the liver tumors did not increase during pregnancy.[14] This clinical observation indicates that in contrast to synthetic sex steroids, naturally occurring steroids, even in extremely high concentrations, can be metabolized easily by the human liver.

OC use is clearly contraindicated in patients with estrogen receptor–positive breast cancer, because pharmacologically active estrogens may stimulate tumor progression.

Major elective surgery is associated with an increased risk of deep venous thrombosis and pulmonary embolism. Therefore it is advisable and is officially recommended to discontinue the use of OCs 4 weeks before a surgical procedure.[15, 16] This recommendation, however, has been questioned by others.[17] Emergency surgery with subsequent immobilization of the patient requires immediate discontinuation of the pill; prophylactic heparin treatment should be considered.[18]

Relative contraindications to the use of OC are listed in Table II. Migraine should be considered a relative contraindication, because epidemiologic studies indicate an increased incidence of headache attacks during the intake of OCs.[17] In addition, patients tend to be at increased risk of thrombotic stroke. If, however, migraine is temporally related to menstrual bleeding, OCs can be prescribed. A tricyclic regimen is recommended, and the patient is advised to take the pill continuously for 3 months, followed by a 7-day pill-free interval.[19, 20]

Patients suffering from focal migraine caused by transitory cerebral ischemia requiring long-term ergotamine medication should refrain from the use of OCs.[19]

The administration of OCs to diabetic patients has been discussed extensively in the literature. Numerous clinical studies are related to this subject, because it was anticipated that orally active sex steroids might increase peripheral insulin resistance. Whereas exogenous estrogens do not interfere with carbohydrate metabolism, gestagens can reduce cell responsiveness to insulin, leading to slightly elevated circulating insulin levels.[21] The diabetogenic effect of progestogens is caused by a change in insulin receptor binding or a postreceptor defect.[22] Chronically elevated glucose levels lead to inhibition of lipolysis and cholesterol synthesis, resulting in the development of atherogenic lesions. The use of low-dose OCs seems effective in decreasing alterations in carbohydrate metabolism compared with high-dose formulations.[23] However, even low-dose preparations must be considered amplifiers of risk factors.[24] Meanwhile there is general consensus that an otherwise healthy young diabetic patient can use low-dose OCs for a limited duration without being at risk of worsening the metabolic conditions. However, careful monitoring of these patients is required. Vascular lesions related to the diabetic disease are considered absolute contraindications to further use of OCs.

Crohn's disease is also listed as a relative contraindication. According to the data of the Royal College of General Practitioners' cohort study,[25] there is evidence that the incidence of Crohn's disease was increased by 40% in OC users. In addition, in a case-control study,[26] the incidence of Crohn's disease was doubled in women taking OCs. Therefore precautions in prescribing OCs to these patients must be taken.

Epilepsy requires long-term treatment with antiepileptic drugs. All commonly used compounds for the treatment of this disease are considered hepatic enzymes inducers. Therefore increased OCs doses may be necessary.[20] Another alternative is the tricyclic regimen. The frequency of epileptic attacks can be reduced by maintaining steady hormone levels.[20] In addition, it is advisable to reduce the pill-free interval to 4 to 5 days. Furthermore, the cycle should be monitored carefully by keeping a menstrual diary. Breakthrough bleedings indicating enzyme induction require increased doses of ethinyl estradiol.

Sickle cell disease is associated with an increased risk of thrombosis. Therefore OCs should not be used, particularly when other risk factors are present.[27]

REFERENCES

1. Maede T. Oral contraceptives, clotting factors and thrombosis. Am J Obstet Gynecol 1982;142:758-64.
2. Mammen EF. Oral contraceptives and blood coagulation: a critical review. Am J Obstet Gynecol 1982;142:781-5.
3. Sabra A, Bronner J. Hemostatic changes induced by 50 μg and 30 μg estrogen progestogen oral contraceptives. J Reprod Med 1983;28:85-91.
4. Prömpeler H, Wieacker P, Hillemanns HG, Breckwoldt M. Einsatz von GnRH-Analoga bei markumarisierten Patientinnen zur Antikonzeption und Prophylaxe von lebensbedrohlichen Ovulationsblutungen. Geburtsh. Frauenheilk 1988;48:588-9.
5. Hall JG, Pauli RM, Wilson KM. Maternal and fetal sequelae of anticoagulation during pregnancy. Am J Med 1980;68:122-30.
6. Geisthövel F, Jenne D, Wieacker P, Breckwoldt M. Cyclic

administration of an LHRH analogue and of progesterone in risk patients to oral contraceptives. Contraception 1987;37:459-69.
7. Beckerhoff R, Luetscher IA, Wilkinson R, et al. Plasma renin concentration, activity and substrate in hypertension induced by oral contraceptives. J Clin Endocrinol Metab 1972;43:1067-72.
8. Khaw KT, Peart WS. Blood pressure and contraceptive use. Br Med J 1982;285:403-6.
9. Pritchard JA, Pritchard SA. Blood pressure response to estrogen-progesterone oral contraceptives after pregnancy-induced hypertension. AM J OBSTET GYNECOL 1977;129:733-9.
10. Castelli WP, Garrison PJ, Wilson PW, et al. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986;256:2835-8.
11. Upton GV. Lipids, cardiovascular disease and oral contraceptives: a practical perspective. Fertil Steril 1990;53:1-12.
12. Huggins GR, Zucker PK. Oral contraceptives and neoplasia: 1987 update. Fertil Steril 1987;47:733-61.
13. Rooks JB, Ory HW, Ishak KG, Strauss LT. Epidemiology of hepaticocellular adenoma. The role of contraceptive use. JAMA 1978;242:644-9.
14. Breckwoldt M. Hormonale Kontrazeptive: Nutzen-Risiko Geburtsh. Frauenheilk 1989;49:521-2.
15. Guillebaud J. Surgery and the pill. Br Med J 1985;291: 498-9.
16. Guillebaud J. Oral contraceptives in risk groups: exclusion or monitoring. AM J OBSTET GYNECOL 1990;163:443-6.
17. Sue-Ling H, Hughes LE. Should the pill be stopped preoperatively? Br Med J 1988;296:447-8.
18. Guillebaud J. Practical prescribing of the combined oral contraceptive pill. In: Filshie M, Guillebaud J, eds. Contraception science and practice. Butterworths: London, 1989.
19. Benson MD, Rebar RW. Relationship of migraine headache and stroke to oral contraceptive use. J Reprod Med 1986;31:1082-8.
20. Mattson RH, Cramer JA, Darney PD, Naftolin F. Use of oral contraceptives by women with epilepsy. JAMA 1986;256:238-48.
21. Gaspard UJ, Lefebvre PJ. Clinical aspects of the relationship between oral contraceptives, abnormalities in carbohydrate metabolism, and the development of cardiovascular disease. AM J OBSTET GYNECOL 1990;163:334-43.
22. Skouby SO, Andersen O, Petersen KR, Mølsted-Pedersen L, Kühl C. Mechanism of action of oral contraceptives on carbohydrate metabolism at the cellular level. AM J OBSTET GYNECOL 1990;163:343-8.
23. Wynn V. Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. AM J OBSTET GYNECOL 1982;142:739-46.
24. Realini JP, Goldzieher JW. Oral contraceptives and cardiovascular disease: a critique of the epidemiological studies. AM J OBSTET GYNECOL 1985;152:729-98.
25. Royal College of General Practitioners. Oral contraceptives and health an interim report. New York: Pitman, 1974.
26. Lesko SM, Kaufmann DW, Rosenberg L. Evidence for an increased risk of Crohn's disease in oral contraceptive users. Gastroenterology 1985;89:1046-9.
27. Evans DJ. Sickle cells and hormonal contraception. Br J Fam Plann 1984;3:80-1.

Conclusion

Prof. Dr. Herbert Kuhl, MD

Frankfurt am Main, Federal Republic of Germany

The program of the symposium on "Pharmacokinetics of Oral Contraceptives and Drug Interactions" was planned to enable comprehensive discussions between basic scientists and clinicians. Even though the appraisal of the clinical relevance of certain in vitro findings remains controversial, it might induce new insights into the mechanism of action of the pill and the cause of adverse effects.

The meeting began with the pharmacokinetics of steroids contained in modern low-dose pills. The discussion focussed on two points, namely, the large intra- and interindividual variations and the rise in steroid levels during multiple intake. Variability is not limited to steroids undergoing first-pass effect and can be observed to a similar extent when the steroids are administered vaginally. When the serum concentrations are measured several times in the same women, a distinct individual pharmacokinetic pattern becomes discernible. Therefore the interindividual variations are in all probability caused by genetic or acquired differences in the metabolic capacity of the liver and the intraindividual variations by short-term metabolic changes induced by environmental factors. It is interesting to know that even during percutaneous or transdermal estrogen replacement therapy, the serum levels of estradiol and estrone vary to a similar degree within a few hours. Regarding the pill, large variations have also been observed in the ratio between the levels of ethinyl estradiol and the prostogen, indicating that changes in the metabolism of estrogens and progestogens must not be parallel and that there is no optimal estrogen/progestogen ratio. The rise in the serum levels of nortestosterone derivatives during multiple intake is well known and was associated with the binding

Reprint requests: Prof. Dr. Herbert Kuhl, Division of Gynecological Endocrinology, Department of Obstetrics and Gynecology, J. W. Goethe University, Theodor-Stern-Kai 7, D-6000 Frankfurt am Main 70, Federal Republic of Germany.
6/0/23621

of steroids to sex hormone–binding globulin (SHBG), the production of which is increased by ethinyl estradiol. However, some observations have indicated that an inhibition of hepatic metabolism of the progestogens by contraceptive steroids is also involved. The steady-state levels of levonorgestrel and gestodene were higher than those obtained by computer simulation based on single-dose administration. Moreover, during intake of a low-dose combination of 30 μg of ethinyl estradiol and 150 μg of levonorgestrel, the levonorgestrel levels rose, even though the SHBG levels remained unchanged. A similar rise in the serum concentrations was also observed during multiple intake with ethinyl estradiol, which is nearly exclusively bound by albumin. Because albumin is not influenced by the pill and ethinyl estradiol sulfates do not represent a reservoir for ethinyl estradiol on account of a half-life similar to that of ethinyl estradiol, it must be assumed that the increase in the levels of ethinyl estradiol is caused by an inhibition of hepatic metabolism.

Gastrointestinal metabolism plays a role in the pharmacokinetics of steroids undergoing first-pass effect. Ethinyl estradiol is metabolized to a large degree in the gut wall, mainly by sulfate conjugation. Desogestrel and norgestimate, which are prodrugs, are also substantially metabolized in the intestinal tract. The pharmacokinetics of prohormones may differ from that of steroid hormones by the lapse of time until peak levels are reached. While desogestrel is rapidly converted in the gut and liver into the hormonally active metabolite 3-keto-desogestrel, the mechanism of action of norgestimate is less clear, and the pharmacokinetics of levonorgestrel-17-β-acetate, levonorgestrel-3-oxime, and levonorgestrel, which are metabolites of norgestimate, are only partly known. It has to be taken into consideration that a retarded conversion of a prodrug may avoid high peak concentrations of the active hormone in the liver during the first liver passage and consequently may reduce the influence of the hormone on hepatic metabolism.

It is assumed that steroid distribution between various protein-bound and non-protein-bound components in serum may influence their bioavailability at target cells and their elimination rate. Contrary to ethinyl estradiol, which is bound with low affinity to albumin, the nortestosterone derivatives are more or less tightly bound to SHBG. Both levonorgestrel and gestodene are bound with high affinity to SHBG, whereas 3-keto-desogestrel is markedly less and norgestimate and levonorgestrel-3-oxime are not at all bound to SHBG. However, the role of serum protein binding in the control of pharmacokinetics and pharmacodynamics still remains a matter of controversy, because, on the one hand, binding proteins has been suggested to be involved in the process of steroid uptake by target cells, and on the other hand, association to and dissociation from binding proteins is a dynamic process that is markedly influenced by the concentration gradient of the steroid between blood and cell. Consequently, the inactivation rate within the cell and the specific binding to receptors or proteins in the cytoplasm or nucleus play an important role. Nuclear receptor binding of steroids is the principal determinant of biologic action, and because the number of specific receptors is limited, full occupation and maximal response may occur at relatively low hormone concentrations. The involvement of serum protein binding in the control of hepatic inactivation and elimination remains obscure, because levonorgestrel and gestodene are bound by SHBG with similar affinities, but only gestodene accumulates in the blood to such an extent that extraordinarily high serum levels are reached despite the lowest dose contained in an oral contraceptive.

Ethinyl estradiol is predominantly metabolized by hydroxylation of the aromatic ring A. The relevance of irreversible binding of the synthetic estrogen to proteins and deoxyribonucleic acid remains unresolved. The pathway of these chemical reactions, which may also affect hepatic enzymes, is unclear, but oxidative conversion of 2-hydroxy-ethinyl estradiol into a reactive intermediate seems to be involved. Steroids containing an ethinyl group are capable of inactivating hepatic cytochrome P-450-dependent enzymes, which are responsible for oxidation and demethylation of steroids and drugs, such as the 2-hydroxylation of ethinyl estradiol. It was demonstrated in vitro and in vivo that after oxidative conversion of the ethinyl group, the reactive intermediate binds irreversibly to the heme group of the cytochrome that is destroyed. With the use of human liver microsomes, several ethinylated steroids were found to be mechanism-based inactivators (suicide inhibitors) of cytochrome, among which gestodene was the most potent. The chemical reactions of activated intermediates, both at the aromatic ring A and the ethinyl group, may be responsible for the rise in steroid levels during multiple intake of contraceptive steroids. The inhibitory action of high gestodene concentrations, particularly during first liver passage, might be causative for the higher serum concentrations of ethinyl estradiol during treatment with a combination of 30 μg ethinyl estradiol and 75 μg of gestodene compared with those with 30 μg of ethinyl estradiol and 150 μg desogestrel.

Drugs may interact with the pharmacokinetics of steroids by induction of hepatic enzymes, resulting in an enhanced metabolism of contraceptive steroids that may impair contraceptive effectiveness. On the other hand, oral contraceptives may inhibit drug-metabolizing enzymes, resulting in a prolongation of half-life and an increase in the serum concentrations of a series of drugs. The clinical relevance of these effects is not clear, but an enhancement of the adverse

effects of imipramine during concomitant treatment with oral contraceptives has been reported.

Antibiotics may interfere with the enterohepatic recirculation of ethinyl estradiol by destroying gut flora. Because intestinal bacteria may develop resistance to the antibiotic within several days, the effect may be transitory. There is little direct conjugation of progestogens at the 17β-hydroxy group, and the main metabolic steps are A ring reduction and subsequently conjugation of the 3-hydroxy group. Therefore enterohepatic recirculation of progestogens is of no importance. Because in low-dose oral contraceptives the progestogen component is responsible for ovulation inhibition and contraceptive effects on the cervix and endometrium, treatment with antibiotics may cause irregular bleedings by reducing the estrogen level but may hardly impair contraceptive safety, provided that the pill is taken correctly and the antibiotic does not induce steroid-metabolizing enzymes in the liver.

Two aspects must be considered when oral contraceptives are applied in a disease state; the most important problem is the absolute and relative contraindications, for example, in patients with thromboembolic or liver disease in their history, whereas impairment of the contraceptive effect may result from reduced absorption in patients with gastrointestinal diseases. The bioavailability of steroids is not affected by antacid administration, and neither diarrhea of colonic origin nor ulcerative colitis was found to influence absorption. Whether ileostomy or proctocolectomy may reduce bioavailability remains to be clarified. Oral contraceptives are contraindicated in patients with a history of thromboembolic disease, ischemic heart attack, or cerebral stroke. Administration of the pill should be discontinued 4 weeks before elective surgery. During long-term anticoagulant therapy, ovulation should be prevented, since ruptured follicles can cause massive intraperitoneal bleeding. In these patients treatment with a potent luteinizing hormone–releasing hormone analog should be a suitable method. Essential hypertension and liver disease are also contraindications, as well as hypercholesterolemia, which often is associated with atherosclerosis and an increased risk of myocardial infarction.

Pharmacodynamics include both receptor-mediated effects and receptor-independent actions on enzymes and other proteins, on membranes, and so forth. When potent sex steroids are used, receptor-mediated hormonal effects may reach an upper limit at relatively low serum concentrations. In this case it will be difficult to substantiate significant differences in the effect of, for example, different progestogens. A similar problem is the possible effectiveness of endogenous estradiol produced by developing follicles during treatment with low- or high-dose oral contraceptives. Although occupation of a relatively small portion of estrogen receptors may trigger the maximal biologic effect in some target tissues, it cannot be excluded that high estradiol levels may contribute to side effects such as mastalgia. It remains to be clarified whether during the elimination phase of the time-versus-concentration curve with decreasing ethinyl estradiol levels, endogenous estradiol may become effective. On the other hand, mastalgia might also be the result of insufficient action of the progestogen component, which at the same time is the cause of follicular activity.

Contrary to this, receptor-independent effects, such as irreversible interactions of ethinylated steroids with proteins and other functional units, are largely dependent on dose and concentration. They may well explain the toxic effects of ethinylated steroids on the liver during chronic treatment, which has been known for many years.

Although the papers presented at this symposium and the intensive discussions that followed have clarified some questions concerning the action and safety of oral contraceptives, the questions that have newly emerged are more than those that could be answered. The factors responsible for the large day-to-day variations in the steroid levels and the hepatic metabolism remain to be investigated; mental and physical stress, diet, and drugs may be involved. Deficient knowledge also surrounds the role of target cell metabolism and storage in fat tissue. Could ethinyl estradiol be transported in lipoproteins after esterification with fatty acids? Is there a change in the pharmacokinetics with age?

The most important conclusion of the symposium might be that obviously the development of the pill has not yet reached an endpoint, as was claimed after the introduction of the recent low-dose oral contraceptives, and that new concepts are necessary.